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**The use of semiochemicals to enhance the natural control of pests of
arable crops by invertebrate predators**

Dean Kirkland

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Faculty of Science and Engineering
The University of Edinburgh
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Abstract.

The electrophysiological and behavioural responses of the aphidophagous hoverfly *Episyrphus balteatus* Degear, and polyphagous ground beetles *Pterostichus melanarius* Illeger and *Nebria brevicollis* Linnaeus, to a range of volatile semiochemicals were studied. *Phacelia tanacetifolia* flower extract, *Nepeta cataria* whole plant extract, (E)- β -farnesene, and food supplement sprays were found to be attractive to the predators in laboratory and wind tunnel assays.

Field trials demonstrated that the attractive semiochemicals could be placed in dispensers in arable crops to increase the amount of predators present throughout the field system. There was also a corresponding decrease in the number of pest species present in the treated field plots relative to the control plots. *Phacelia* flower extract was found to have the greatest effects on predator and prey numbers.

The mechanisms of the reduction in prey numbers were studied using exclusion experiments in the field and Enzyme linked immunosorbant assays. There was found to be a link between the increased rates of predation in the field and the decline in pest numbers.

Declaration.

This thesis is my own composition, the results presented are of investigations conducted by myself, work other than my own is clearly indicated by reference to relevant workers or their publications and has not been presented in any previous application for a higher degree.

Dean Lee Kirkland.

Dedication.

To all things creepy crawly, the fascination of children with jars.

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Table of contents

	Page
Title page	I
Abstract	II
Dedication	III
Declaration	IV
Acknowledgements	V
Table of contents	VI
List of plates	XIII
List of figures	XIV
List of tables	XXXIV
	Page
Chapter 1: General Introduction to natural control	
and methods	1
1.1 Introduction	2
1.2 Classical biological control methods	4
1.3 Predators and parasitoids present in arable fields and	
their potential to control pests	8
1.4 Aims of the project	12

Chapter 2: The use of semiochemicals in natural	
pest control	13
2.1 Introduction to semiochemicals	14
2.2 Choice of semiochemicals used in the study	16
2.2.1 Natural honeydews, components and artificial foods	16
2.2.2 Green leaf volatiles	18
2.2.3 <i>Phacelia tanacetifolia</i> Benth	20
2.2.4 <i>Nepeta cataria</i> Linneaus, nepetalactol and nepetalactone	20
2.2.5 Synthetic insect pheromones	21
2.3 Techniques used in semiochemical research	22
2.4 Production of semiochemicals used in the study	26
2.4.1 Introduction	26
2.4.2 <i>Phacelia</i> and <i>Nepeta</i> plant extracts	26
2.4.3 Tenax-Ta extraction and GC-MS analysis of <i>Phacelia</i> extract	28
2.4.4 (E)- β -farnesene	32
2.4.5 Other semiochemicals	34
Chapter 3: Predators selected for the study	35
3.1 <i>Carabidae</i>	36
3.1.1 <i>Pterostichus melanarius</i> Illeger	41
3.1.2 <i>Nebria brevicollis</i> Fabricius	43

3.2	The hoverfly <i>Episyrphus balteatus</i>	
	Degear (Diptera: Syrphidae)	45
3.3	Ladybirds <i>Coccinnella septempunctata</i>	
	Linneaus and <i>Adalia bipunctata</i> Linneaus	
	(Coleoptera: Coccinellidae)	54
3.4	Provision of insect material	60
3.4.1	Aphids <i>Myzus persicae</i> and <i>Sitobion</i>	
	<i>avenae</i>	60
3.4.2	Carabidae <i>Pterostichus melanarius</i>	
	and <i>Nebria brevicollis</i>	61
3.4.3	<i>Episyrphus balteatus</i> culture	62
3.4.4	Coccinellids <i>Coccinnella septempunctata</i>	
	and <i>Adalia bipunctata</i>	63
	Chapter 4: Electrophysiological response to semiochemicals	65
4.1	Introduction	66
4.2	Materials and methods	67
4.2.1	Analysis of data	73
4.3	Results	76
4.3.1	<i>P. melanarius</i>	76
4.3.2	<i>N. brevicollis</i>	93
4.3.3	<i>E. balteatus</i>	93

	Page
4.4 Discussion	94
4.5 Summary	97
 Chapter 5: Olfactometry	98
5.1 Introduction	99
5.2 Materials and methods	100
5.2.1 Semiochemicals	104
5.2.2 Statistical analysis of data	105
5.3 Results	106
5.4 Discussion	116
5.5 Summary	119
 Chapter 6: Wind tunnel assays	120
6.1 Introduction	121
6.2 Materials and methods	122
6.3 Results	125
6.4 Discussion	130
6.5 Summary	133
 Chapter 7: Field season 1996 and 1997	134
7.1 Introduction	135
7.2 Materials and methods	138

	Page
7.2.1 Field studies 1996	138
7.2.2 Field studies 1997	144
7.2.3 Statistical analysis	147
7.3 Results	148
7.3.1 1996	148
7.3.1.1 Winter wheat	148
7.3.1.2 Spring barley	150
7.3.2 1997	160
7.4 Discussion	176
7.4.1 1996	176
7.4.2 1997	179
7.5 Summary	182
 Chapter 8: Mechanisms of reduction of pest numbers	183
8.1 Introduction	184
8.1.1 Assessing predation	186
8.2 Materials and methods	188
8.2.1 Field trials	188
8.2.2 Statistical analysis of data	191
8.2.3 Enzyme linked immunosorbent assays (ELISAs)	191
8.2.4 Statistical analysis of ELISA data	197

	Page
8.3 Results	199
8.3.1 Field studies 1998	199
8.3.2 ELISA tests of carabid gut contents	204
8.4 Discussion	215
8.4.1 Field studies 1998	215
8.4.2 ELISA	217
8.5 Summary	219
 Chapter 9: Summary and discussion	 221
9.1 Discussion of techniques	222
9.2 Electrophysiological response of predators	226
9.3 Behavioural response of predators	228
9.4 Field responses	230
9.5 Potential for use in IPM systems	233
9.6 Future work	234
Bibliography	236
 Appendices	 307
Appendix 1. EAG P values	308
Appendix 2. P values from behavioural studies	309
Appendix 3. P values from 1996 field studies	

Appendix 4. Numbers of Coccinellids and Syrphids

	Page
trapped in 1996 and 1997	311
Appendix 5. P values from field studies in 1997	312
Appendix 6. P values for aphids trapped in the treated plots in 1997	314
Appendix 8. P values For ELISAs	315
Publications arising from this study	316
Manipulating the behaviour of beneficial insects in cereal crops to Enhance control of aphids	317
Aromatherapy for crops: Enhancing natural control of grain aphids in cereals	324

List of plates

	Page
Chapter 3.	
Plate 3.1 <i>Pterostichus melanarius</i> Illiger	44
Plate 3.2 <i>Nebria brevicollis</i> Fabricius	44
Plate 3.3 <i>Episyrphus balteatus</i> Degear	47
Plate 3.4 <i>Coccinnella septempunctata</i> Linneaus	53
Plate 3.5 <i>Adalia bipunctata</i> Linneaus	55

List of Figures

Page

Chapter 2.

Fig. 2.1 Diagram of (E)- β -farnesene synthesis equipment 33

Chapter 4.

Fig 4.1 The EAG experimental design (modified from Evans, 1991) 68

Fig. 4.2 The EAG response in mV (\pm SE) of *P. melanarius*
to Acetaldehyde (n=12) 77

Fig. 4.3 The EAG response in mV (\pm SE) of *P. melanarius* to
artificial aphid honeydew (n=12) 77

Fig. 4.4 The EAG response in mV (\pm SE) of *P. melanarius* to
 β -caryophyllene (n=12) 78

Fig. 4.5 The EAG response in mV (\pm SE) of *P. melanarius* to
 β -hydroxybutyric acid (n=12) 78

Fig. 4.6 The EAG response in mV (\pm SE) of *P. melanarius* to
L-tryptophan (n=12) 79

Fig. 4.7 The EAG response in mV (\pm SE) of *P. melanarius* to
Phacelia leaf extract (n=12) 79

Fig. 4.8 The EAG response in mV (\pm SE) of <i>P. melanarius</i> to <i>Nepeta</i> extract (n=12)	80
Fig. 4.9 The EAG response in mV (\pm SE) of <i>P. melanarius</i> to cis-3-hexanol (n=12, * = Threshold concentration)	80
Fig 4.10 The EAG response in mV (\pm SE) of <i>P. melanarius</i> to <i>Phacelia</i> flower extract (n=12, * = Threshold concentration)	81
Fig 4.11 The EAG response in mV (\pm SE) of <i>P. melanarius</i> to (E)- β -farnesene (n=10, * = Threshold concentration)	81
Fig. 4.12 The EAG response in mV (\pm SE) of <i>N. brevicollis</i> to acetaldehyde (n=12)	82
Fig. 4.13 The EAG response in mV (\pm SE) of <i>N. brevicollis</i> to artificial aphid honeydew (n=12)	82
Fig. 4.14 The EAG response in mV (\pm SE) of <i>N. brevicollis</i> to β -caryophellene (n=12)	83

	Page
Fig. 4.15 The EAG response in mV (\pm SE) of <i>N. brevicollis</i> to β -hydroxybutyric acid (n=12)	83
Fig. 4.16 The EAG response in mV (\pm SE) of <i>N. brevicollis</i> to <i>Phacelia</i> leaf extract (n=12)	84
Fig. 4.17 The EAG response in mV (\pm SE) of <i>N. brevicollis</i> to <i>Phacelia</i> flower extract (n=12)	84
Fig. 4.18 The EAG response in mV (\pm SE) of <i>N. brevicollis</i> to L-tryptophan (n=12)	85
Fig. 4.19 The EAG response in mV (\pm SE) of <i>N. brevicollis</i> to cis-3-hexanol (n=12, * = Threshold concentration)	85
Fig. 4.20 The EAG response in mV (\pm SE) of <i>N. brevicollis</i> to (E)- β -farnesene (n=12, * = Threshold concentration)	86
Fig. 4.21 The EAG response in mV (\pm SE) of <i>N. brevicollis</i> to <i>Nepeta</i> extract (n=10, * = Threshold concentration)	86
Fig. 4.22 The EAG response in mV (\pm SE) of <i>E. balteatus</i> to artificial aphid honeydew (n=12)	87

Fig. 4.23 The EAG response in mV (\pm SE) of <i>E. balteatus</i> to β -caryophellene (n=12)	87
Fig. 4.24 The EAG response in mV (\pm SE) of <i>E. balteatus</i> to β -hydroxybutyric acid (n=12)	88
Fig. 4.25 The EAG response in mV (\pm SE) of <i>E. balteatus</i> to L-tryptophan (n=12)	88
Fig. 4.26 The EAG response in mV (\pm SE) of <i>E. balteatus</i> to <i>Phacelia</i> leaf extract (n=12)	89
Fig. 4.27 The EAG response in mV (\pm SE) of <i>E. balteatus</i> to (E)- β -farnesene (n=12, * = Threshold concentration)	89
Fig. 4.28 The EAG response in mV (\pm SE) of <i>E. balteatus</i> to <i>Phacelia</i> flower extract (n=12, * = Threshold concentration)	90
Fig. 4.29 The EAG response in mV (\pm SE) of <i>E. balteatus</i> to cis-3-hexanol (n=12, * = Threshold concentration)	90

Fig. 4.30 The EAG response in mV (\pm SE) of <i>E. balteatus</i> to <i>Nepeta</i> extract (n=10, * = Threshold concentration)	91
---	----

Fig. 4.31 The EAG response in mV (\pm SE) of <i>E. balteatus</i> to Acetaldehyde (n=10, * = Threshold concentration)	91
--	----

Chapter 5.

Fig. 5.1 The four armed olfactometer (Tréfás <i>et al.</i> , In Press)	102
--	-----

Fig. 5.2 Mean time (\pm SE) male <i>P. melanarius</i> spent in each of the odour fields (n=20) in the four armed olfactometer with no test semiochemical	108
---	-----

Fig. 5.3 Mean time (\pm SE) female <i>P. melanarius</i> spent in each of the odour fields (n=20) in the four armed olfactometer with no test semiochemical	108
---	-----

Fig. 5.4 Mean time (\pm SE) male <i>P. melanarius</i> spent in each of the odour fields (n=20) in the four armed olfactometer with (E)- β -farnesene as the test semiochemical (* = $P < 0.05$)	109
--	-----

Fig. 5.5 Mean time (\pm SE) female <i>P. melanarius</i> spent in each of the odour fields (n=20) in the four armed olfactometer with (E)- β -farnesene as the test semiochemical	109
--	-----

Fig. 5.6 Mean time (\pm SE) male <i>P. melanarius</i> spent in each of the odour fields (n=20) in the four armed olfactometer with <i>Nepeta</i> extract as the test semiochemical	110
Fig. 5.7 Mean time (\pm SE) female <i>P. melanarius</i> spent in each of the odour fields (n=20) in the four armed olfactometer with <i>Nepeta</i> extract as the test semiochemical	110
Fig. 5.8 Mean time (\pm SE) male <i>P. melanarius</i> spent in each of the odour fields (n=20) in the four armed olfactometer with <i>Phacelia</i> flower extract as the test semiochemical (* = $P < 0.05$)	111
Fig. 5.9 Mean time (\pm SE) female <i>P. melanarius</i> spent in each of the odour fields (n=20) in the four armed olfactometer with <i>Phacelia</i> flower extract as the test semiochemical (* = $P < 0.05$)	111
Fig. 5.10 Mean time (\pm SE) male <i>N. brevicollis</i> spent in each of the odour fields (n=20) in the four armed olfactometer with no test semiochemical	112
Fig. 5.11 Mean time (\pm SE) female <i>N. brevicollis</i> spent in each of the odour fields (n=20) in the four armed olfactometer with no test semiochemical	112

Fig. 5.12 Mean time (\pm SE) male *N. brevicollis* spent in each of the odour fields (n=20) in the four armed olfactometer with (E)- β -farnesene as the test semiochemical (* = $P < 0.05$) 113

Fig. 5.13 Mean time (\pm SE) female *N. brevicollis* spent in each of the odour fields (n=20) in the four armed olfactometer with (E)- β -farnesene as the test semiochemical 113

Fig. 5.14 Mean time (\pm SE) male *N. brevicollis* spent in each of the odour fields (n=20) in the four armed olfactometer with *Nepeta* extract as the test semiochemical 114

Fig. 5.15 Mean time (\pm SE) female *N. brevicollis* spent in each of the odour fields (n=20) in the four armed olfactometer with *Nepeta* extract as the test semiochemical 114

Fig. 5.16 Mean time (\pm SE) male *N. brevicollis* spent in each of the odour fields (n=20) in the four armed olfactometer with *Phacelia* leaf extract as the test semiochemical 115

Fig.5.17 Mean time (\pm SE) female <i>N.brevicollis</i> spent in each of the odour fields (n=20) in the four armed Olfactometer with <i>Nepeta</i> extract as the test semiochemical	115
--	-----

Chapter 6.

Fig.6.1 Diagrammatic representation of the wind tunnel used in this study (modified from Hern, 1997)	123
Fig 6.2 Mean % of male <i>E. balteatus</i> (\pm SE) carrying out specified behaviour in the wind tunnel with acetaldehyde as an odour source (n=3)	126
Fig 6.3 Mean % of female <i>E. balteatus</i> (\pm SE) carrying out specified behaviour in the wind tunnel with acetaldehyde as an odour source (n=3)	126
Fig 6.4 Mean % of male <i>E. balteatus</i> (\pm SE) carrying out specified behaviour in the wind tunnel with (E)- β -farnesene as an odour source (n=3)	127
Fig 6.5 Mean % of female <i>E. balteatus</i> (\pm SE) carrying out specified behaviour in the wind tunnel with (E)- β -farnesene as an odour source (n=3)	127

Fig 6.6 Mean % of male <i>E. balteatus</i> (\pm SE) carrying out specified behaviour in the wind tunnel with <i>Phacelia</i> flower extract as an odour source (n=3)	128
--	-----

Fig 6.7 Mean % of female <i>E. balteatus</i> (\pm SE) carrying out specified behaviour in the wind tunnel with <i>Phacelia</i> flower extract as an odour source (n=3)	128
--	-----

Fig 6.8 Mean % of male <i>E. balteatus</i> (\pm SE) carrying out specified behaviour in the wind tunnel with <i>Nepeta</i> extract as an odour source (n=3)	129
--	-----

Fig 6.9 Mean % of female <i>E. balteatus</i> (\pm SE) carrying out specified behaviour in the wind tunnel with <i>Nepeta</i> extract as an odour source (n=3)	129
--	-----

Chapter 7

Fig. 7.1 Field layout in 1996	142
-------------------------------	-----

Fig. 7.2 The semiochemical dispenser	143
--------------------------------------	-----

Fig. 7.3 Field layout in 1997	146
-------------------------------	-----

Fig. 7.4 Mean number of carabids (\pm SE) trapped in winter wheat in 1996 with artificial honeydew treatment (n=3)	151
---	-----

Fig. 7.5 Mean number of carabids (\pm SE) trapped in winter wheat in 1996 with L-tryptophan treatment (n=3)	151
Fig. 7.6 Mean number of carabids (\pm SE) trapped in winter wheat in 1996 with (E)- β -farnesene treatment (n=3)	152
Fig. 7.7 Mean number of carabids (\pm SE) trapped in winter wheat in 1996 with <i>Nepeta</i> extract treatment (n=3)	152
Fig. 7.8 Mean number of carabids (\pm SE) trapped in winter wheat in 1996 with <i>Phacelia</i> flower extract treatment (n=3)	153
Fig. 7.9 Mean number of aphids (\pm SE) trapped in winter wheat in 1996 with artificial honeydew treatment (n=3)	153
Fig. 7.10 Mean number of aphids (\pm SE) trapped in winter wheat in 1996 with L-tryptophan treatment (n=3)	154
Fig. 7.11 Mean number of aphids (\pm SE) trapped in winter wheat in 1996 with (E)- β -farnesene treatment (n=3)	154

Fig. 7.12 Mean number of aphids (\pm SE) trapped in winter wheat in 1996 with <i>Nepeta</i> extract treatment (n=3)	155
Fig. 7.13 Mean number of aphids (\pm SE) trapped in winter wheat in 1996 with <i>Phacelia</i> flower extract treatment (n=3)	155
Fig. 7.14 Mean number of carabids (\pm SE) trapped in spring barley in 1996 with Envirofeast™ treatment (n=3)	156
Fig. 7.15 Mean number of carabids (\pm SE) trapped in spring barley in 1996 with (E)- β -farnesene treatment (n=3)	156
Fig. 7.16 Mean number of carabids (\pm SE) trapped in spring barley in 1996 with <i>Nepeta</i> extract treatment (n=3)	157
Fig. 7.17 Mean number of carabids (\pm SE) trapped in spring barley in 1996 with <i>Phacelia</i> flower extract treatment (n=3)	157
Fig. 7.18 Mean number of aphids (\pm SE) trapped in spring barley in 1996 with Envirofeast™ treatment (n=3)	158

Fig. 7.19 Mean number of aphids (\pm SE) trapped in spring barley in 1996 with (E)- β -farnesene treatment (n=3)	158
Fig. 7.20 Mean number of aphids (\pm SE) trapped in spring barley in 1996 with <i>Nepeta</i> extract treatment (n=3)	159
Fig. 7.21 Mean number of aphids (\pm SE) trapped in spring barley in 1996 with <i>Phacelia</i> flower extract treatment (n=3)	159
Fig. 7.22 Mean numbers of carabids (\pm SE) trapped per week in each field in 1997 as predicted by randomised linear model (n=17)	161
Fig. 7.23 Mean numbers of carabids (\pm SE) trapped per week at each distance in 1997 as predicted by randomised linear model (n=17)	161
Fig. 7.24 Mean numbers of aphids (\pm SE) trapped per week in each field in 1997 as predicted by randomised linear model (n=17)	162
Fig. 7.25 Mean numbers of carabids (\pm SE) trapped per week at each distance in 1997 as predicted by randomised linear model (n=17)	162

Fig. 7.26 Mean number of carabids (\pm SE) trapped at 0-10m in 1997 with (E)- β -farnesene treatment (n=3)	163
Fig. 7.27 Mean number of carabids (\pm SE) trapped at 45-55m in 1997 with (E)- β -farnesene treatment (n=3)	163
Fig. 7.28 Mean number of carabids (\pm SE) trapped at 90-100m in 1997 with (E)- β -farnesene treatment (n=3)	164
Fig. 7.29 Mean number of carabids (\pm SE) trapped at 0-10m in 1997 with Envirofeast™ treatment (n=3)	164
Fig. 7.30 Mean number of carabids (\pm SE) trapped at 45-55m in 1997 with Envirofeast™ treatment (n=3)	165
Fig. 7.31 Mean number of carabids (\pm SE) trapped at 90-100m in 1997 with Envirofeast™ treatment (n=3)	165
Fig. 7.32 Mean number of carabids (\pm SE) trapped at 0-10m in 1997 with <i>Nepeta</i> extract treatment (n=3)	166

Fig. 7.33 Mean number of carabids (\pm SE) trapped at 45-55m in 1997 with <i>Nepeta</i> extract treatment (n=3)	166
Fig. 7.34 Mean number of carabids (\pm SE) trapped at 90-100m in 1997 with <i>Nepeta</i> extract treatment (n=3)	167
Fig. 7.35 Mean number of carabids (\pm SE) trapped at 0-10m in 1997 with <i>Phacelia</i> flower extract treatment (n=3)	167
Fig. 7.36 Mean number of carabids (\pm SE) trapped at 45-55m in 1997 with <i>Phacelia</i> flower extract treatment (n=3)	168
Fig. 7.37 Mean number of carabids (\pm SE) trapped at 90-100m in 1997 with <i>Phacelia</i> flower extract treatment (n=3)	168
Fig. 7.38 Mean number of aphids (\pm SE) trapped at 0-10m in 1997 with (E)- β -farnesene treatment (n=3)	169
Fig. 7.39 Mean number of aphids (\pm SE) trapped at 45-55m in 1997 with (E)- β -farnesene treatment (n=3)	169

Fig. 7.40 Mean number of aphids (\pm SE) trapped at 90-100m in 1997 with (E)- β -farnesene treatment (n=3)	170
Fig. 7.41 Mean number of aphids (\pm SE) trapped at 0-10m in 1997 with Envirofeast™ treatment (n=3)	170
Fig. 7.42 Mean number of aphids (\pm SE) trapped at 45-55m in 1997 with Envirofeast™ treatment (n=3)	171
Fig. 7.43 Mean number of aphids (\pm SE) trapped at 90-100m in 1997 with Envirofeast™ treatment (n=3)	171
Fig. 7.44 Mean number of aphids (\pm SE) trapped at 0-10m in 1997 with <i>Nepeta</i> extract treatment (n=3)	172
Fig. 7.45 Mean number of aphids (\pm SE) trapped at 45-55m in 1997 with <i>Nepeta</i> extract treatment (n=3)	172
Fig. 7.46 Mean number of aphids (\pm SE) trapped at 90-100m in 1997 with <i>Nepeta</i> extract treatment (n=3)	173

	Page
Fig. 7.47 Mean number of aphids (\pm SE) trapped at 0-10m in 1997 with <i>Phacelia</i> flower extract treatment (n=3)	173
Fig. 7.48 Mean number of aphids (\pm SE) trapped at 45-55m in 1997 with <i>Phacelia</i> flower extract treatment (n=3)	174
Fig. 7.49 Mean number of aphids (\pm SE) trapped at 90-100m in 1997 with <i>Phacelia</i> flower extract treatment (n=3)	174
 Chapter 8	
Fig. 8.1 Diagramatic representation of the ELISA assay	187
Fig. 8.2 Field Layout 1998	190
Fig. 8.3 The ELISA Plate	195
Fig. 8.4 Mean number (\pm SE) of carabids trapped in the <i>Phacelia</i> flower extract treated non-barriered plots relative to control non-barriered plots (n=3)	200
Fig. 8.5 Mean number (\pm SE) of carabids trapped in controlled barriered plots relative to the control non-barriered plots (n=3)	200

Fig. 8.6 Mean number (\pm SE) of carabids trapped in the <i>Phacelia</i> barriered relative to control non-barriered plots (n=3)	201
Fig. 8.7 Mean number (\pm SE) of carabids trapped in the <i>Phacelia</i> barriered relative to non-barriered plots (n=3)	201
Fig. 8.8 Mean number (\pm SE) of aphids trapped in <i>Phacelia</i> flower extract treated non-barriered plots relative to control non-barriered plots (n=3)	202
Fig. 8.9 Mean number (\pm SE) of aphids trapped in controlled barriered plots relative to the control non-barriered plots (n=3)	202
Fig. 8.10 Mean number (\pm SE) of aphids trapped in the <i>Phacelia</i> barriered plots relative to control non-barriered plots (n=3)	203
Fig. 8.11 Mean number (\pm SE) of aphids trapped in the <i>Phacelia</i> barriered plots relative to non-barriered plots (n=3)	203
Fig. 8.12 Mean percentage (\pm SE) of <i>P. melanarius</i> trapped from each field in 1996 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3)	205

Fig. 8.13 Mean percentage (\pm SE) of <i>P. melanarius</i> trapped from each treatment in 1996 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3)	205
Fig. 8.14 Mean percentage (\pm SE) of <i>P. melanarius</i> trapped each week in 1996 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3)	206
Fig. 8.15 Mean percentage (\pm SE) of <i>N. brevicollis</i> trapped from each field in 1996 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3)	206
Fig. 8.16 Mean percentage (\pm SE) of <i>N. brevicollis</i> trapped from each treatment in 1996 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3)	207
Fig. 8.17 Mean percentage (\pm SE) of <i>N. brevicollis</i> trapped from each week in 1996 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3)	207
Fig. 8.18 Mean percentage (\pm SE) of <i>P. melanarius</i> trapped from each field in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3)	208

- Fig. 8.19 Mean percentage (\pm SE) of *P. melanarius* trapped from each distance in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3) 208
- Fig. 8.20 Mean percentage (\pm SE) of *P. melanarius* trapped from each treatment in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3) 209
- Fig. 8.21 Mean percentage (\pm SE) of *P. melanarius* trapped from each week in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3) 209
- Fig. 8.22 Mean percentage (\pm SE) of *N. brevicollis* trapped from each field in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3) 210
- Fig. 8.23 Mean percentage (\pm SE) of *N. brevicollis* trapped from each distance in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3) 210

Fig. 8.24 Mean percentage (\pm SE) of <i>N. brevicollis</i> trapped from each treatments in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3)	211
Fig. 8.25 Mean percentage (\pm SE) of <i>N. brevicollis</i> trapped from each week in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3)	211
Fig. 8.26 Mean percentage (\pm SE) of <i>P. melanarius</i> trapped from each field in 1998 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3)	212
Fig. 8.27 Mean percentage (\pm SE) of <i>P. melanarius</i> trapped from each treatment in 1998 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3)	212
Fig. 8.28 Mean percentage (\pm SE) of <i>P. melanarius</i> trapped from each week in 1998 with significant ($\geq 91 \mu\text{l/ml}^{-1}$) levels of prey proteins in their guts (n=3)	213
Fig. 8.29 Mean percentage (\pm SE) of <i>N. brevicollis</i> trapped from each field in 1998 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3)	213

Fig. 8.30 Mean percentage (\pm SE) of *N. brevicollis* trapped from each treatment in 1998 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3) 214

Fig. 8.31 Mean percentage (\pm SE) of *N. brevicollis* trapped from each week in 1998 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (n=3) 214

List of tables

	Page
Chapter 2	
Table 2.1 Main components of <i>Phacelia</i> flower extract volatile mixture	31
Chapter 3	
Table 3.1 Cues for oviposition in <i>E. balteatus</i> (modified from Chandler, 1966)	52
Chapter 4	
Table 4.1 Order of stimuli used in EAG studies	71
Table 4.2 List of volatile compounds screened for EAG response of <i>Pterostichus melanarius</i> , <i>Nebria brevicollis</i> and <i>Episyrphus balteatus</i>	72
Table 4.3 EAG values for a female <i>E. balteatus</i> to <i>Phacelia</i> Flower extract	75
Table 4.4 Threshold concentrations for <i>P.</i> <i>melanarius</i>	92
Table 4.5 Threshold concentrations for <i>N.</i> <i>brevicollis</i>	92
Table 4.6 Threshold concentrations for <i>E.</i> <i>balteatus</i>	92

	Page
Chapter 5	
Table 5.1 Randomised linear model for olfactometer data	107
Chapter 7	
Table 7.1 Randomised linear model for field data analysis	149
Chapter 8	
Table 8.1 The Genstat program for the analysis of field data	192
Table 8.2 The Genstat linear model for analysis of ELISA data	198
Appendix 1	
Table 10.1 EAG P values for <i>P. melanarius</i> (n=6)	308
Table 10.2 EAG P values for <i>N. brevicollis</i> (n=6)	308
Table 10.3 EAG P values for <i>E. balteatus</i> (n=6)	308
Appendix 2	
Table 10.4 P values for <i>P. melanarius</i> in the four armed olfactometer (n=20)	309

	Page
Table 10.5 P values for <i>N. brevicollis</i> in the four armed olfactometer (n=20)	309
Table 10.4 P values for <i>E. balteatus</i> in the wind tunnel (n=3)	309
Appendix 3	
Table 10.7 P values for carabids trapped per week in the 1996 field season (n=3)	310
Table 10.8 P values for carabids trapped per field in the 1996 field season (n=3)	310
Table 10.9 P values for aphids trapped per week in the 1996 field season (n=3)	310
Appendix 4	
Table 10.10 Total numbers of Coccinellids and Syrphids trapped in 1996 and 1997	311
Appendix 5	
Table 10.11 P values from field studies in 1997 (n=3)	312
Table 10.12 P values for carabids in treated plots in 1997 (n=3)	312
Table 10.13 P values for aphids trapped in 1997 (n=3)	312

	Page
Table 10.14 P values for aphids trapped in treated plots in 1997 (n=3)	313

Appendix 6

Table 10.15 P values for carabids trapped in 1998 field season	314
--	-----

Table 10.16 P values for aphids trapped in 1998 field season	315
--	-----

Appendix 7

Table 10.17 P values for aphid protein from Carabids trapped in 1996	315
--	-----

Table 10.18 P values for slug protein from Carabids trapped in 1996	315
---	-----

Table 10.19 P values for aphid protein from Carabids trapped in 1997	315
--	-----

Table 10.20 P values for slug protein from Carabids trapped in 1997	315
---	-----

Table 10.21 P values for aphid protein from Carabids trapped in 1998	315
--	-----

Table 10.22 P values for slug protein from Carabids trapped in 1998	315
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Chapter 1

General introduction to natural control methods

Chapter 1. General introduction to natural control methods

1.1 Introduction

Each year invertebrate pests such as aphids, slugs, flies and weevils cause severe economic damage to arable crops. Despite the use of 2.5 million tons of pesticide each year world-wide, approximately 35% of world crop production is lost to pests, with an additional 20% lost to pests post harvest (Pimentel *et al.*, 1992). For example in just one crop, oilseed rape, a very valuable economic crop with over 4,000 hectares planted in Britain each year (Pickett, 1995) is host to a variety of pest species. One of these species the peach-potato aphid *Myzus persicae* Sultzer can cause a 30% loss of seed yield (Buntin, 1995). The cabbage aphid, *Brevicoryne brassicae* Linneaus, a more specialised *Brassica* feeder, can cause up to a 77% reduction in crop yield and an 11% reduction in seed oil content (Kelm & Godomski, 1995). The fecundity and movements of pest species can also be very high, and it has been reported that one aphid species, *Therioaphis trifolii* Monell, was able to spread over an area of 50,000 km² in less than a year (Hofsvang, 1990).

Pests are usually controlled by the use of persistent chemical insecticides, however this has several disadvantages. Although there have been widespread changes in the pesticides used since the highly persistent organochlorine insecticides widely used in the 1940s through to the 1970s, pesticide residues are still a major contaminant of land and water sources in rural areas (Knight & Cooper, 1996).

In fact, throughout the world pesticide contamination of land and water has increased significantly over the past twenty years, with over 130 different contaminants identified in ground water (Cohen *et al.*, 1996).

Pesticide residues are passed up the food chain and have been found in the tissues of different groups of wildlife (Fossi *et al.*, 1995, Mansingh *et al.*, 1997). Even when levels of pesticides were sublethal they have been shown to cause changes in behaviour, and longevity of various animal groups (Davies & Cook, 1993, Tasei *et al.*, 1994, Liess & Schulz, 1996).

Residues of many pesticides have been found to be contaminants of human food. In a study in Australia, the levels of these residues were generally low, both DDT and HCH were found to exceed Australian government safe limits (Kannan *et al.*, 1994). A similar pattern is also seen in studies from India. (Kannan *et al.*, 1992). Pesticides have also been shown to be accumulating within human tissues. Samples of human tissue from autopsies in Poland revealed high levels of contamination by pesticide residues (Czaja *et al.*, 1993). Pesticide residue contaminants have also been detected in human breast milk and have been found to be passed on from mother to infant (Rodriguez-Cordoba *et al.*, 1983; Alomar *et al.*, 1986).

Despite the drawbacks outlined above, pesticides are still widely used throughout world agriculture, but only manage to save approximately 10% of world food production (Pimentel *et al.*, 1992). This proportion is falling each year due to problems of increased resistance amongst pest populations. In one study in Florida 25 examples of varying degrees of pesticide resistance were found in fields of use ranging from agricultural, horticultural, medical, veterinary and household pests

(Leibee & Capinera, 1995). With problems of resistance in two important economic pests the leafminer *Liriomyza trifolii* Burgess and the diamondback moth *Plutella xylostella* Linneaus, resistance problems have become so great that it severely limits pest management options (Leibee & Capinara, 1995). Pesticide resistance in the past few years has increased rapidly and is now much more extensive and widespread than has been previously predicted (Roush, 1993).

If effective pest control is to be maintained in the future and the problems of contamination and pollution to be overcome, alternative methods of pest control must be used.

1.2 Classical biological control methods

Biological control is defined as the artificial control of pests and parasites by use of other organisms (Abercrombie *et al.*, 1980). Introduced arthropods as biological control agents have been used successfully for many years in greenhouse crops for the control of pests such as the glasshouse whitefly, *Trialeurodes vaporariorum* Westwood by the parasitoid *Encarsia formosa* Gahan (Van Lenteren *et al.*, 1996; Van Roermund *et al.*, 1997). However, until recently this approach has not been feasible in a field situation (Harwood *et al.*, 1992). The scale of introduction necessary to produce an economic level of control combined with the fact that insects are highly mobile and with no way to ensure that the introduced insect will remain in the area, make this an unsuitable method of control.

Biological control of insect pests relying on the introduction of natural enemies from elsewhere has only had limited success in the field. Between two thirds to three quarters of all introduced organisms fail to become established (Greathead, 1995). Furthermore, only 16% of projects involving introduced species have been completely successful in controlling the target pest (Hall *et al.*, 1980).

Various proposals have been put forward to explain the failure of introduced organisms to become established including, insufficient adaptation in introduced species to enable them to cope with prevailing climatic conditions, insufficient genetic variation in introduced predator populations, and too low numbers of organisms released (Hopper *et al.*, 1995). Other hypotheses have also been suggested as to why even when an introduced predator becomes established it does not always achieve satisfactory levels of control. For example, poor phenological synchronicity with the pest, temperature ranges for activity of the predator more restricted than the pest, poor searching ability of the natural enemy, low host suitability, ephemeral habitats with pest having higher colonising abilities than the predator, and insufficient aggregation of the predators in high pest densities (Hopper *et al.*, 1995).

The Citrus whitefly, *Dialeurodes citri* Ashmead, is an extremely serious pest of citrus groves from Florida to Texas, causing a loss of 45-50% of the value of the crop (Rosen & Debach 1991). In looking for a biological control agent it was soon discovered that although the whitefly was present on citrus crops throughout Asia its population densities were so low it was not considered a pest. This was found to be due to the actions of a Coccinellid *Cryptognatha flavescens* Motshulsky and a parasitoid *Encarsia lahorensis* Howard (Rosen & Debach, 1991). However, although

the attempt was made several times, no *C. flavescens* ever survived the trip to the USA. Although *E. lahorensis* did survive the trip and populations rapidly established in the laboratory, when they were released into the field the pest populations were all at an inappropriate developmental stage for parasitism and so parasitoid populations could not become established, and were lost (Rosen & Debach, 1991).

In studies in the USA any attempt by scientists to introduce captive bred individuals of the ladybirds *Harmonia axyridis* Pallas and *Propylea quatuordecimpunctata* Linneaus (Coleoptera: Coccinellidae) failed, and they quickly disappeared from the areas of artificial introduction. It was only when the insects came into the country on cargo ships and established themselves naturally, did they begin to spread and become effective control agents (Bauer, 1995).

So biological control in a field situation has mainly concentrated on the use of microbial pesticides, insect viruses and bacteria and bacterial toxins formulated and manufactured so they can be sprayed directly onto the crop. These can be contact microbials which penetrate the insect's cuticle after the insect contacts a treated surface, this type tend to be fungi, but need relatively high humidity conditions (Badilla and Alves, 1991). Or they may be ingested microbials; a desiccation resistant stage of the agent can be sprayed which is ingested along with the food and causes massive disruption to the insect gut (Baines *et al.*, 1997). Spores of the naturally occurring soil bacterium *Bacillus thuringiensis* (BT) are used extensively for this purpose. The spores can be sprayed directly to the crop and ingested, or toxin genes can be incorporated in phage to be applied to plants (Marzari *et al.*, 1997), or

the plants themselves can be genetically modified to incorporate the toxin producing genes (Hokkanen & Wearing 1994; Schell, 1997).

Microbial pesticides have several advantages over traditional chemical insecticides, they are highly target specific, leave no toxic residues, only require a low dosage, are fully compatible with chemical control and only lead to resistance slowly (Van Emden, 1991).

However, they also have some distinct disadvantages. Careful timing of the sprays may be needed as they may affect a certain stage of the insect. They are also so specific that some developmental stages of the pest may be virtually immune to them. Some microbial pesticides need a certain threshold population level of pests before the disease can spread. The agent may also lose virulence through the production process, although there are no toxic residues the rotting corpses of dead insects are left behind on the crops. The agent may rely on damp conditions, and have limited dispersal, and the widespread use of large amounts of pathogens with a high mutation rate and close relation to human pathogens could cause severe difficulties (Van Emden, 1991).

Even though resistance to microbial toxins only develops slowly in pest populations, resistance is beginning to develop. Resistance to the BT toxin first appeared in the diamond back moth *P. xylostella* a global vegetable pest. Populations of the moth in the continental United States, Hawaii and several Asian countries have developed resistance to the toxin. Resistance in the moth is controlled by a recessive gene which is associated with reduced binding of the toxin to the larval midgut membrane (Tabashnik *et al.*, 1996). Since 1985 the potential for resistance has been

demonstrated in 5 pest species, and can develop to very high levels in only a few generations (McGaughey, 1994).

Because of these problems with pesticide contamination and resistance there has recently been increased interest in the use of predators naturally present throughout agricultural systems.

1.3 Predators and parasitoids present in arable fields and their potential to control pests

Predators and parasitoids have a very large potential to control pest populations. Although adults of the green lacewing, *Chrysopa carnea* Stephens (Neuroptera: Chrysopidae), are not predatory and feed exclusively on nectar and pollen, each *C. carnea* larva will consume on average 554 aphids between hatching and maturation to adulthood (Singh & Singh, 1994a). Similarly the larvae of the hoverfly *Episyrphus balteatus* Degear (Diptera: Syrphidae) will each consume 508 aphids on average before pupation occurs (Singh & Singh, 1994b).

Other insects could cause even greater reductions in the number of pests, for example the Coccinellidae, as both the larvae and adults are predatory. The 1st to 4th instar of *Coccinella septempunctata* larvae will take on average 624 aphids apiece, while the adults may eat another 140 (Singh & Singh, 1994b).

Each female *C. carnea* produces a mean of 477 eggs, the female *Metasyrphus corrales* Fabricius hoverfly 436 eggs with a generation time of approximately three weeks, and females of the two spot ladybird *Adalia bipunctata* Linnaeus will

produce a mean of 1466 eggs during her lifetime (Hofsvang, 1990). Therefore predators have the potential to make a real contribution to the control of pests within the arable field system, however modern agricultural practices and uneven distribution of predators within fields reduces the contribution that predators make to pest control.

For example, in Egyptian wheat fields experimental plots were established which excluded all Coccinellids in a particular area and aphid numbers were allowed to build up. After two weeks Coccinellids were reintroduced into half of the plots at approximately the same relative abundance that occurred naturally in other wheat fields. This led to a significant decrease in the numbers of aphids in the plots and an increase in yield of 46-49% in the experimental plots relative to the control plots (El Hag, 1992). In the United States introduction of an exotic Asian ladybird *H. axyridis* led to a significant reduction in the numbers of pecan growers who had to spray to control the pecan aphid, *Melanocallis caryaefoliae* Davis, in the areas where it was introduced (Bauer, 1995).

A variety of management practices can be used to enhance the number of predators within arable fields (Wyss, 1995). Weed strips planted around the field margins can lead to an increase in the number of predators in the field. Field trials in Switzerland have demonstrated that in orchards, sowing weed strips between the trees can significantly increase the number of aphidophagous predators, and reduce the number of aphids throughout the orchard as compared to traditionally managed orchards (Wyss, 1995). This is thought to be because the weed strips provide protection, overwintering sites and resources for the predators that are not available

inside the crop (Lys *et al.*, 1994). Similarly studies in the UK have revealed that the number of aphidophagous *E. balteatus* adults and eggs was significantly increased in unsprayed field headlands as compared to herbicide treated headlands, as unsprayed headlands supported a more diverse range of plants which provided greater resources for the predators (Cowgill, 1993).

Intercropping of the crop can also significantly increase the number of predators amongst the crop. Weed strips planted amongst a winter wheat crop lead to a significant increase in the activity density of carabids in the crop adjacent to the weed strips (Lys & Nentwig, 1994). Intercropping corn and soy beans increased the number of most foliage dwelling predators (Tonhasca, 1993). Female carabid beetles from intercropped fields were found to be significantly heavier, with better filled crops than from monoculture fields. Also there seemed to be an elongation of the reproductive period of female carabids in intercropped fields (Lys & Nentwig, 1994). However, the use of predators for biocontrol also has several disadvantages; because of modern intensive agricultural practices the number of predators present in arable fields may be relatively low, and predators are also unevenly distributed throughout arable field systems (Jepson, 1984). Pesticide treatments and fragmentation of agricultural habitats which removes overwintering habitats and other non-prey resources has begun to lead to cases of local extinction of predators from agricultural land (Jepson, 1984).

In a study of cereal fields in East Anglia, spraying of pesticides was shown to reduce both the total number of individual *Carabidae* present, and the species diversity of

carabids in the field (Hawthorne & Hassal, 1994). The use of herbicides to control agricultural weeds can also have deleterious effects on the composition of the predator community. In some predator species the adults feed exclusively on nectar and pollen, while other organisms such as ladybirds still require a carbohydrate source to provide the energy needed for behaviours such as flight. Use of herbicides reduces the number of plants able to provide these resources. (Cowgill *et al.*, 1994).

Populations of predators are generally not homogeneously distributed throughout the crop (Dennis, 1991). In the spring carabids migrate into the crop from the field margins where they overwinter, however it takes time for them to penetrate through the entire crop. Early in the season the majority of predators are only to be found in the first 1-10m away from the field margin (Dennis, 1991), but if predators are to carry out effective control of pests, then they have to penetrate the crop early in the season before large pest populations can become established.

Even when weed strips and intercropping are used to increase the number of predators there can be problems. Studies on carabids in intercropping systems have revealed that responses can be very species specific with numbers of some species increased while others are actually reduced (Tonhasca, 1993). Intercropping soybean and maize actually led to an increase in some species of herbivore in the crop (Tonhasca, 1993). There is also a significant fall in the number of predators caught as distance away from the weed strips declined.(Lys *et al.*, 1994).

1.4 Aims of the project

The aim of this research project is to encourage predator movement into crops by the use of semiochemicals which alter their behaviour, and to demonstrate that the predators have an impact on pest populations.

Several techniques will be used to examine this:-

- Electrophysiological studies, to identify the semiochemicals that induce an antennal response in the predators.
- Olfactometry, to identify the behavioural response of ground dwelling predators to the semiochemicals.
- Wind tunnel assays, to qualify behavioural response of flying predators to the semiochemicals.
- Field studies, to determine the effect of the semiochemicals on both predator and aphid populations at all distances throughout the field system
- Enzyme linked immunosorbant assays (ELISAs), to determine the relationship between predator and pest populations in the field.

Chapter 2

The use of semiochemicals in natural pest control

Chapter 2. The use of semiochemicals in natural pest control

2.1 Introduction to semiochemicals

Some of the problems outlined in the previous chapter may be overcome through the use of semiochemicals, an umbrella term used to describe both toxins and information conveying chemicals (or infochemicals) which induce specific behaviours in insects (Nordlund & Lewis, 1976). Chemicals produced by plants and insects are known to play a major role in the behavioural events that determine the success or failure of insects (Renwick, 1992).

Infochemicals can be divided into two main groups. The first are pheromones which mediate interactions between individuals of the same species, and allelochemicals, information bearing chemicals that mediate interactions between individuals of different species (Roitberg & Isman, 1992). Most chemicals used by predators fall into the latter category (Roitberg & Isman, 1992).

Allelochemicals can be further subdivided into three categories depending on the balance of costs and benefits:

- Allomones where the emitter benefits at cost to the receiver. For example, the bolas spiders, *Mestophora* species, emit an analogue of the sex pheromone of some female *Lepidoptera*, used to attract male moths to where they can be easily caught (Stowe *et al.*, 1995).
- Synomones where both the emitter and receiver benefit. This is exemplified by the various plant compounds used by predators and parasitoids to locate the habitat of their prey or hosts. The predator benefits by obtaining food while the plant benefits

through the loss of pest species (Dwumfour, 1992; Agelopoulos, 1994; Turlings *et al.*, 1995).

Kairomones, where the receiver benefits at cost to the emitter. Many of the compounds produced by the prey organism, but used as location cues by the predator fall into this category. For example, in many aphid species the female emits a sex pheromone that is used to attract males for mating. For many species the active components of this pheromone have been identified as nepetalactol and nepetalactone (Dawson *et al.*, 1990). Synthetic analogues of this pheromone can be produced in the laboratory and used to disrupt aphid mating systems (Dawson *et al.*, 1982). However, this compound is also highly attractive to several parasitoid species (Dawson *et al.*, 1990). Parasitoids use the semiochemical to locate their prey and so benefit, while the aphid which emits the pheromone is eaten.

The specificity and non-toxic nature of semiochemicals makes them an appealing option for use within an integrated pest management program (Roitberg & Isman, 1992). Semiochemicals are used to control pests in several different ways, for example sex pheromones can be employed in traps to lure in and kill pest species, pest population monitoring, and for mating disruption (Roitberg & Isman, 1992). While alarm pheromones can be employed to drive pests from the area (Dawson *et al.*, 1982). However, emphasis in previous studies has been on semiochemicals which affect pest behaviour. This study aims to identify semiochemicals which affect predator behaviour.

2.2 Choice of semiochemicals used in the study

Much evidence suggests that odours are extremely important in mediating tritrophic level interactions (Whitman 1988). Although the majority of the studies on host and prey odour attraction have involved parasitoids (Eller *et al.*, 1988; Ding *et al.*, 1989; Grasswitz & Paine, 1992 & 1993; Agelopoulos & Keler, 1994, Geervliet *et al.*, 1994), a variety of different chemicals have been identified as being highly attractive to predators. In olfactometer trials, certain members of the *Carabidae* have been shown to orient to air flows passed over prey species before entering the olfactometer chamber (Wheater *et al.*, 1989; Kielty *et al.*, 1996).

Coccinella septempunctata Linneaus respond to volatile kairomones in air passed over four species of aphids. First to fourth instar larvae were attracted to kairomones from all four aphid species equally. However, the adults showed definite preferences, being most strongly attracted to *M. persicae*, then to *Aphis fabae* Scopoli, *Rhopalosiphum pisum* Harris, and *Rhopalosiphum maidis* Fitch (Sengonca & Lui, 1993). Larvae also showed some orientation to volatiles produced by other individuals of the same instar, giving some indication of an aggregation factor produced by the larvae which help others locate prey (Sengonca & Lui, 1993).

2.2.1 Natural honeydews, components and artificial foods

The choice of semiochemicals used in the study was based on compounds identified from the literature, the reasons for the choice are detailed below. Aphids secrete a sticky solution rich in carbohydrates from their cornicles, this is honeydew. Honeydew is very similar to nectar in that its chemical composition is almost identical to that of

plant sap (Evans, 1995). This may provide predators with a supplementary energy source along with the more widely used nectar and pollen (Evans, 1995).

In an early study 246 insect species belonging to 50 families were found to consume aphid honeydew, and of these 66% were aphidophagous (Evans, 1995). Large increases in longevity have been recorded in insects taking aphid honeydews, and adding aphids to field plots attracted in polyphagous predators looking for honeydew, which then reduced the number of other pest species in the plot (Evans, 1995).

However, it is impractical to obtain large quantities of honeydew from aphids, so artificial substitutes have been created consisting of a mixture of yeast extract, sucrose and water in a ratio of 4: 7: 10. Addition of this artificial honeydew to field plots significantly increased the numbers of predators in those plots, particularly *Coccinellidae*, *Syrphidae*, and *C. carnea* in both cotton (Nichols & Neel, 1977) and potatoes (Ben Saad & Bishop, 1976).

Two major components of natural aphid honeydews are acetaldehyde and L-tryptophan. The breakdown products of acid hydrolysed L-tryptophan have been shown to be highly attractive to the green lacewing *C. carnea* in both arable crops (Hargen *et al.*, 1986) and in olive tree canopies (McEwen *et al.*, 1994). Acetaldehyde is also produced by a fungus found in rotting fruit and from yeast fermentation products and is used as a location cue for *Biosteres longicaudatus* Ashmead, a Tephritid fruit fly parasitoid (Greany *et al.*, 1977) and *Leptopilina heterotoma* Thompson, a larval parasitoid of *Drosophila* (Dicke *et al.*, 1994).

Recently, the formulation of artificial food compounds was improved with the development of Envirofeast™, a food supplement based on a mixture of yeast proteins

and sugars (Mensah, 1997). The supplement has been used successfully to suppress oviposition by lepidopteran pests and to attract parasitoids to cotton crops in Australia (Mensah, 1997).

2.2.2 Green leaf volatiles

Some predators have been shown to orientate to synomones from their host food plants. The predatory flower bug *Anthocoris nemorum* Linneaus, was shown to orientate to volatiles in air passed over leaves from stinging nettles, goat willow and tomato (Dwumfour, 1992). The chemicals released from these plants that caused orientation in the insects were found to be in the green leaf volatile mixture.

Further investigations of green leaf volatiles revealed them to be six carbon alcohols, aldehydes and derivative esters (Visser *et al.*, 1979; Hamilton-Kemp *et al.*, 1989). Green leaf volatiles are released or produced when plant tissue is cut or damaged (Tollsten & Bergström, 1985) and are responsible in part for the odour of damaged leaves (Whitman & Eller, 1990).

As well as reacting to green leaf volatiles released passively by the plant, the plants themselves can actually play an active role in the attraction of predators. Some plants change the composition of the volatiles they release when attacked by pest species in order to attract more predators (Turlings *et al.*, 1995).

The parasitoid *Diaeretiella rapae* McIntosh was found to be attracted to volatile odours from its prey *B. brassicae* and *Diaraphis noxia* Kurdjumov, but would not orientate to volatiles from undamaged host food plants such as cabbage or wheat. Mechanically damaged plants were also unattractive to the parasitoid (Reed *et*

al., 1995). However, when these plants were damaged by the aphids they were found to be highly attractive to the parasitoid. This suggests that the plants are able to change the composition of their volatile mixture in order to attract predators and parasitoids to the plant when it's under attack by pest species (Reed *et al.*, 1995).

The parasitoid *Cotesia rubecula* Marshall uses the larvae of the cabbage white butterfly *Pieris rapae* Linnaeus as its host. In wind tunnel assays the parasitoid orientated to volatiles produced by the host larvae, but volatiles from either mechanically damaged or caterpillar damaged cabbage plants were shown to be more attractive to the parasitoid than host larval volatiles (Geervliet *et al.*, 1994).

The coccinellid *Chilocorus nigritus* Fabricius, which preys on the potato scale insect orientated to volatiles from both the scale insect and its host food plant. However, the beetle spent less time orientating toward the host plant volatiles than toward a mixture of scale insect and potato volatiles or to scale insect volatiles alone (Ponsonby & Copland, 1995).

Cis-3-hexanol is a very common green leaf volatile found in a variety of plants (Whitman & Eller, 1990; Jakobson *et al.*, 1994). Volatile mixtures collected from mechanically and pest damaged cowpea plants were found to contain cis-3-hexanol (Whitman & Eller, 1990). When this isolated compound was tested against female parasitoids (*Micropolitis croceipes* Cresson, Hymenoptera: Braconidae) in a wind tunnel the wasps showed positive orientation to the compound (Whitman & Eller, 1990).

Damaged cotton leaves have been shown to release a variety of green leaf compounds, one major constituent of this green leaf volatile mixture was found to be the terpenoid

β -caryophellene (Ndiege *et al.*, 1991). This compound was shown to be highly attractive to the predators *C. carnea* (Ndiege *et al.*, 1991), and the parasitoid *Campoletis sonorensis* Cameron (Elzen *et al.*, 1984).

2.2.3 *Phacelia tanacetifolia* Benth

Some plants produce volatiles that are attractive to predators not because they are the food plants of prey species, but because they provide other non-prey resources such as nectar and pollen. Predators often show a preference for different plants when feeding. In two separate studies the gut contents of syrphids were examined and indicated that both *E. balteatus* (Cowgill, 1993) and *Melanostoma fasciatum* Macquart (Hickman, 1995) took large quantities of pollen from *Phacelia tanacetifolia* Benth. Sowing strips of *Phacelia* next to the crop not only increased the number of syrphids up to 100m into the crop, but also increased the numbers of *Ichneumonidae* in the crop (Holland *et al.*, 1994). However the attraction was thought to be due to visual cues such as the colours of the flowers or shape of the plant (Hickman, 1995). *Phacelia* was included initially in the study to determine if there is a volatile component to this attraction.

2.2.4 *Nepeta cataria* Linneaus, nepetalactol and nepetalactone

Some work has concentrated on specific single compounds that cause long range attraction in predatory insects. Males and females from 2 species of aphid *B. brassicae* and *Schizaphis borealis* Rondani were tested for responses to volatiles produced by the opposite sex. Females showed no attraction to volatiles from male

aphids, however the males were highly attracted to volatiles from unmated females (Pettersson, 1970).

Using gas chromatography coupled to mass spectroscopy the active components of these sex pheromones were found to be two monoterpenoids, nepetalactol and nepetalactone (Dawson *et al.*, 1987; Herrbach, 1992). These compounds were found to attract a high proportion of males of the hop aphid *Phorodon humili* Schrank to baited traps in hop gardens, achieving aphid control through disruption of mating (Dawson *et al.*, 1990).

The compound nepetalactol was found to be a major component of the green leaf volatile mixture of cat mint *Nepeta cataria* Linneaus. Females of the parasitoid *Praon vulcre* Haliday were shown to be highly attracted to traps baited with nepetalactol, obtained from the plant, between the months of June to November when aphid mating is known to take place (Tumlinson *et al.*, 1991; Hardie *et al.*, 1994; Lilley *et al.*, 1994b).

2.2.5 Synthetic insect pheromones

When aphids are attacked by predators or parasitoids the aphids in the surrounding area initiate one of two possible behaviours. They either stop feeding and begin to walk away from the danger area (Elagamy & Haynes, 1992), or they fall off the plant they are feeding on (Chau & Mackauer 1997). These behaviours provide an indication that the attacked aphid releases some form of volatile alarm pheromone that diffuses through the air to other aphids in the surrounding area, warning them of the presence of danger (Dawson, 1987).

In the early 1970s the alarm pheromone was identified for several aphid species , and in all cases the active component was found to be the sequesterpene (E)- β -farnesene (Herrbach, 1992). In olfactometer assays *C. septempunctata* was highly attracted to (E)- β -farnesene (Nakumata, 1991) as was the parasitoid *Cotesia margineventris* Cresson (Turlings *et al.*, 1992b).

2.3 Techniques used in semiochemical research

A variety of techniques can be used to study the chemical ecology of predators. Electroantennograms or EAGs can be carried out to establish the response of the insect's antenna to volatile compounds (Sazonov, 1988). The insect head is excised and placed between two ring filled electrodes and an air stream containing volatile chemicals is passed over the head (Visser, 1979; Evans & Allen-Williams, 1992). The EAG response recorded is the sum of the responses of all the olfactory cells on the insect's antenna (Schneider, 1963), rather than a recording from a single receptor cell. This is because plant extracts consist of a mixture of volatile compounds, which would stimulate more than one receptor on the antenna. This technique has been used to identify the pheromone/ kairomone/ synomone relationships of several predator prey systems (Ramachandron & Norris, 1991; Vet *et al.*, 1990).

Electroantennograms establish if an insect detects a particular compound, however, they do not reveal what behavioural response the insect will initiate when it detects the compound: i.e. if the insect will be attracted to the compound, or if the insect will be repelled away from the compound. To discover this behavioural assays must be employed.

For behavioural assays on insects which seldom fly, a variety of olfactometers can be used. An olfactometer is a device which presents the insects with one or more test odours, and the behaviour of the insect when it detects the odour can be monitored

A range of different olfactometers have been used to study the behaviour of insects;

- Y and T-tube olfactometers, a simple glass tube with two short arms on the end either at 45° and 90° respectively. An electric pump draws test odour into one arm of the olfactometer and purified air into the other. The insects are released into the end of the glass tube, and the insects move down the tube at the end and have to make a decision about which arm of the olfactometer to enter. The number of insects each arm can then be statistically analysed (Wickremasinghe & Van Emden, 1992; Yasuda *et al.*, 1996). This type of olfactometer is best suited to very small walking and flying insects such as midges (Blackwell *et al.*, 1997).
- The linear track olfactometer, which consists of a vertical tube with a wire running through the centre connecting an upper and lower chamber. Insects are placed in the lower chamber and an electric pump draws the test odour into the upper chamber. To reach the odour in the upper chamber the insects must climb the wire. The number of insects in each of the chambers is then analysed (Hori & Komatsu 1997). This type of olfactometer is used for insects which climb such as cockroaches (Sakuma & Fukami, 1985).
- The four arm olfactometer, which consists of a star shaped chamber with test odour drawn into each arm of olfactometer creating four distinct diamond shaped odour fields. The insect is placed into the centre of the chamber and makes a choice of odour field (Pham-Deluge *et al.*, 1990; Kaiser & Dejong, 1995).

The present study used the four arm olfactometer design as large species of carabids do not climb well (Luff, 1987), therefore the linear track olfactometer is unsuitable for use with carabids. The Y and T tube designs also only present the insect with a single choice between test odour and control.

Although olfactometers are suitable for assessing the behavioural response of insects which forage by walking along the ground, the size of the olfactometer chamber virtually precludes flight (Evans, 1991). Therefore, for large flying insects a wind tunnel assay had to be employed to gauge behavioural response to a range of wind-borne odour sources. An electric fan creates a moving stream of the test odour through a chamber, the insects are introduced into the chamber and their behaviour monitored (Hern 1997; Takken *et al.*, 1997).

Using this technique it has been shown that pest species orientate toward distant sources of host plant odour (Blackmer & Phelan 1992; Zhang & McEvoy, 1995), and to sex and aggregation pheromones emitted from other members of the same species (Vickers & Baker 1994). Parasitoids and predators have also been shown to orientate toward odours associated with host and prey species (Shu *et al.*, 1990; Noldus *et al.*, 1990 Noldus *et al.*, 1991. Doolittle *et al.*, 1991).

Once attractive chemicals have been identified in the laboratory, they need to be tested in the field. The attractive chemical can be incorporated into some form of chemical dispenser and placed into field plots (Hesler & Sutter, 1993; Rieske & Raffa, 1993), and the plots sampled at specific intervals. Carabids beetles can be sampled by pitfall trapping, which does have its limitations. The technique does not capture all species of invertebrates equally; some species are more susceptible to capture in pitfall traps than

others, and this susceptibility can vary with season and even sex of the insect. This method of capture frequently underestimates the actual populations and variability of invertebrates in the area as compared to absolute sampling techniques, as it only represents the activity/ density of the captured beetles (Topping & Sunderland, 1992; Van Den Berghe, 1992). However absolute sampling methods for ground dwelling invertebrates require either the destruction and removal of large areas of the crop by a D-vac or removal of large quantities of surface soils, for soil washing techniques (Schotzko & O'Keefe, 1989; Spence & Niemela, 1994).

Syrphids, Coccinellids and Chrysopids need to be sampled using sweep nets as they are highly mobile, excellent flyers, and they generally occur in the crop canopy rather than on the ground (Michels & Behle, 1992; Kharboutli & Mack, 1993), although coccinellid larvae have been known to cross the ground in search of new host food plants. Coloured sticky traps would just concentrate the insects already in the plot at the trap, and would therefore not show any increase in predator numbers due to semiochemicals.

Once organisms have been captured in the field they can be preserved by freezing. Later, the gut contents of the frozen insects can be analysed by Enzyme Linked Immunosorbant Assays (ELISA) to assess what the predators have been feeding on (Hagler & Naranjo, 1994). The results of this can then be used to quantify the amount of predation of various pest species taking place in plots containing semiochemical dispensers (Sopp *et al.*, 1992). The ELISA technique also enables the amount of prey protein in the guts of individual predators to be quantified, giving greater information about predation rates of the predators on particular prey species. Other biochemical

methods used previously to study predation only provide information on consumption rates (Sunderland, 1994).

2.4 Production of semiochemicals used in the study

2.4.1 Introduction

Yeast autolysate (Sigma-Aldrich Chemical Company Ltd, Gillingham, UK) was mixed with sucrose (Sigma-Aldrich Chemical Company Ltd, Gillingham, UK) and distilled water in a ratio of 4: 7: 10 yeast: sucrose: water. The mixture was stirred magnetically at 50°C until all of the yeast and sucrose had dissolved. The artificial honeydew was stored at 4°C.

Envirofeast™, an experimental insect diet (Mensah, 1997), used in some of the field trials was kindly supplied by Rhône-Poulenc Agrochemicals Ltd, Ongar Research Centre, Essex UK.

2.4.2 *Phacelia* and *Nepeta* plant extracts

Phacelia and *Nepeta* seeds were obtained from Chiltern Seeds (Ulverston, Cumbria). 30 x 20cm perforated plastic seed trays were filled with peat (John Innes Irish moss peat), watered and left for a few minutes for the water to soak into the compost. *Nepeta cataria* seed was then sprinkled thinly over the surface of the compost, so that one packet (approximately 2g) provided enough seed for two seed trays. The seed trays were placed in a 1.8m x 1m x 0.7m nylon mesh covered wooden framed cage. The mesh front of the cage was held on by velcro fastening to allow easy access for watering. The mesh was fine enough to prevent aphids or any larger insect from

gaining access to the plants. This ensured that the plants did not have to be sprayed with insecticide, preventing contamination of the plant extracts with pesticide residues that may affect the behaviour of the insects in the later trials.

The cage was located inside a glass house with lighting provided by 8 halogen lamps located on the roof of the glasshouse and set on a timer to provide a 18: 6 hour light dark regime. Hot water pipes running through the glasshouse maintained a constant temperature of 20 ± 2 °C with heat sensors connected to a motor controlling the ceiling vents, which opened if the temperature rises above 20 °C.

The plants were watered three times weekly. When large enough to be handled without damaging them the seedlings were transferred into individual 15cm diameter plant pots filled with peat. The plants were allowed to grow until fully mature and just before flowering the plants were harvested, the main stems were cut with a pair of scissors just above the soil surface.

Phacelia tanacetifolia plants were maintained under the same conditions as the *Nepeta* plants in a cage in the glasshouse. However *Phacelia* seeds were sown in 45cm diameter 40cm tall perforated plastic garden tubs. The tubs were three quarters filled with peat and watered. the seed was then sprinkled on the surface of the compost with one packet (approximately 2g) providing enough seed for three tubs. Four 1m tall garden canes were placed in the tubs with garden twine looped round them to provide support for the mature plants. The plants were watered three times per week. The flowers and leaves of the mature plants were harvested by cutting the base of the stem with a pair of scissors.

Once the plant material was harvested it was weighed and placed into a blender (Philips 019681 DBS). Methanol was added to the plant material in a ratio of 1: 10 weight of plant material to volume of methanol. The mixture was blended for 1 minute to produce a very finely blended mixture.

The mixture was filtered under reduced pressure in a buchner flask and pump (Vacuum pump compressor DPC8LV, Genevac, Ipswich, UK). The liquid fraction collecting in the bottom of the flask was retained. The filtrate was placed in a conical flask, the neck sealed with parafilm™ and stored at 4°C in a spark proof fridge.

2.4.3 Tenax-Ta extraction and GC-MS analysis of *Phacelia* extract

The aims of the Tenax-Ta extraction and GC-MS analysis of *Phacelia* extract were to identify the major components of the volatile mixture from *Phacelia tanacetifolia* flowers. Tenax extraction of *Nepeta cataria* was not needed as volatiles from *N. cataria* have already been identified in previous studies (Ceyrolles & Tamada, 1999). Although the volatile composition of *Nepeta* extract may vary due to prevailing environmental conditions such as mean temperature and daylength, the main components, nepetalactol and nepetalactone, will remain the same although their proportions may vary slightly (Ceyrolles & Tamada, 1999).

A Tenax-Ta column was prepared by placing 0.5g of Tenax-Ta (Alltech Associates, Carnforth, Lancashire, UK) in clean glass disposable 5ml Pasteur pipettes, packing each end with glass wool to prevent the Tenax-Ta falling through the pipette. The Tenax-Ta columns were conditioned using a disposable 10ml plastic syringe (Plastipak, Becton Dickinson, Dublin, Ireland) to pass 10ml of hexane through the

Tenax-Ta. The Tenax-Ta columns were stored at room temperature in an airtight glass container until use.

100g of fresh plant material was placed in a 100ml conical flask containing approximately 50ml of distilled water, which was placed at the bottom of a clean air tight cylindrical gas jar 20cm tall 10cm diameter. The lid of the jar was replaced and sealed with sealing tape (PTFE thread seal tape, Phase Separations, Deeside, Clywd, UK). The Tenax-Ta column was placed in one of the sockets in the lid of the jar and was connected to an air flow meter (Platon Air Products, UK) and electric pump (B85 SE, Charles Austin Pumps Ltd, Byfleet, Surrey, UK) via clear PVC tubing, the joints sealed with sealing tape. Another socket on the lid of the jar was connected to two metal filters containing activated charcoal, and the joints sealed with tape. All the other sockets in the lid of the jar were sealed with glass stoppers. Air was drawn through the activated charcoal to remove any volatiles and into the gas jar with the plant material, passed through the Tenax-Ta on the way to the pump. Air flow was regulated to 500 ml/min using the air flow meter. The equipment was left running for 24 hours with each Tenax-Ta column. The glass jar was cleaned at the end of every run, and fresh plant material used for each Tenax-Ta column.

The Tenax-Ta columns were stored in an airtight plastic container. The container was stored at 2°C to prevent evaporation of the volatiles from the Tenax-Ta.

GC-MS analysis was carried out by Dr A. Hern at, ETH Zentrum, Zurich, Switzerland. Volatiles were eluted from the Tenax-Ta columns using 1000µl of methanol. The samples were concentrated by placing 100µl of the methanol sample in a glass vial which was held under a gentle stream of high purity nitrogen (purity 99.999%) until

20µl remained. The samples were then injected into the chromatograph. The instrument was a Hewlett Packard gas chromatograph (HP 6890 + Series) fitted with an auto sampler (HP 7683) and mass selective detector (HP5973). The column used was a Hewlett Packard HP1 (cross linked methyl silicone gum), column length 30m; id 0.25mm; film thickness 0.25µm; and phase ratio 250. The gas chromatograph (GC) was run in constant pressure mode with an injection pressure of 9.00 torr. This produced a column flow of helium (purity 99.999%) of 1.1 ml/min at 50°C. The injection temperature was 200°C, the oven was set at 50°C for 2 minutes, then the temperature was increased by 5°C per minute up to 250°C (held for 2 minutes). The column effluent was transferred to the GC-MS detector via a transfer line (280°C), the Mass spectrometer employed was Hewlett Packard (HP 6795).

Putative identifications were made by searching the National Institute of Standards and Technology mass spectra Library (NIST 98), after the background ions were removed from the mass spectra for each integrated peak and an authentic library of standards. The main components of the *Phacelia* flower extract are shown in Table 2.1.

2.4.4 (E)-β-farnesene

(E)-β-farnesene was produced by the modified method of Dawson *et al.* (1982), in a dehydration reaction from a (E)-nerolidol precursor. 150g of neutral 150 mesh aluminium oxide catalyst (Sigma-Aldrich Chemical Co. Ltd, Gillingham, UK) was placed in a 2cm diameter 33cm long glass chromatography tube with a filter at the

Table 2.1 Main components of *Phacelia* flower extract volatile mixture

Compound	Synonym
Acetic acid (butyl ester)	n-Butyl acetate Butyl ethanoate
Formic acid (hexyl ester)	n-Hexyl formate n-Hexyl methanoate
1-Hexanol	—
2-Butoxy ethanol	β -Butoxy ethanol Butyl glycol Butyl oxitol
Benzaldehyde	Oil of bitter almonds
Acetic acid (hexyl ester)	n-Hexyl acetate Hexyl ethanoate
1-Octanol	—
Benzoic acid (methyl ester)	Clorius Methyl benzoate
Furanmethanol, 5-ethenyl tetrahydrate	Furfuryl alcohol cis-Linalool oxide cis Linalyl oxide
Phenylethyl alcohol	Benzeneethanol β - Hydroxyethyl benzene
Pyridinium, 1-amino-methylchloro	3-Picoline β -Picoline
Decanol	—
Indole	—
Benzoic acid 2-amino (methyl ester)	Anthranilic acid Methyl anthranilate
Benzyl tiglate	—
Bicyclo[5.3.0] decane, 2-methylene-5-butyl	Phthalic acid
Hexadecanoic acid	Adipic acid Bisoflex Doa

bottom to prevent the catalyst falling through. The top of the column was packed with glass wool.

The column was placed in a vertical furnace and held in place and supported using clamps and bosses so that approximately the top and bottom 5cm of the column protruded from the furnace (Fig. 2.1). The top of the furnace was sealed around the top of the column with a heat proof ceramic disk which prevented convection currents from forming in the furnace, and therefore produced an even temperature all over the surface of the column.

A glass adapter was placed in the top of the column connected to a 100ml glass pressure equalising flask with a tap at the bottom. The flask was filled with 100ml of (E)-nerolidol (Sigma-Aldrich Chemical Co. Ltd, Gillingham, UK). A glass adapter with a tap was placed in the top of the pressure equalising flask. This was connected to a length of rubber tubing at the end of which was another adapter connected to a 5ml round bottom glass flask, held onto the adapter by a plastic tab. The flask was filled with 1.5g of pyridine (Sigma Aldrich Chemical Co. Ltd, Gillingham, UK).

The bottom of the flask was connected to a glass T adapter at the bottom of which was a 100ml glass round bottom flask clamped in place. The cross piece of the T adapter was connected to a trap that was immersed in liquid nitrogen so that any residue which passes out of the system was trapped and frozen so that it did not pass onto the pump. The trap was connected to a glass pressure line. The line passed

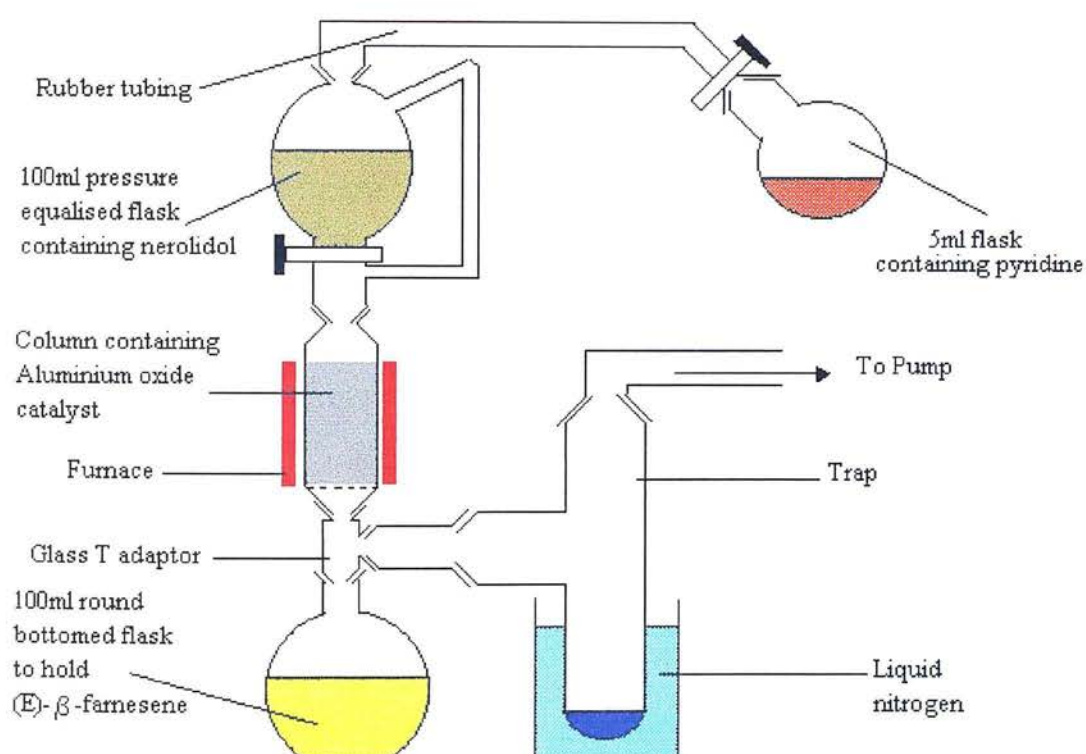


Fig. 2.1 (E)-β-farnesene synthesis equipment (modified from Dawson, *et al.*, 1982).

through another liquid nitrogen cooled trap before entering the pump (EB3A, Edwards Vacuum Components, Crawley, UK). The furnace heated the system to 200°C and the pump reduced the pressure in the system to 0.1 torr. The tap at the bottom of the flask containing pyridine was opened and the pyridine evaporated and passed through the aluminium oxide catalyst. If the pyridine was slow to evaporate it could be encouraged with a hot air gun aimed over the flask.

Once all of the pyridine had evaporated the tap on the bottom of the pressure equalising flask was opened slightly to allow the (E)-nerolidol to drip slowly through the system over approximately 45 minutes, until all the nerolidol had passed through the column. The (E)- β -farnesene collected in the round bottom flask at the bottom of the column. Once all of the (E)- β -farnesene had been collected the system was flushed with nitrogen gas, which brings the system up to normal atmospheric pressures. Nitrogen is used for this process because unlike the oxygen in air it will not form explosive free radicals at the temperature of the liquid nitrogen.

The resulting liquid was analysed by Nuclear Magnetic Resonance by Dr H. McNab, Edinburgh University Department of Chemistry, revealing that it consisted of a mixture of farnesene isomers but was approximately 75% pure (E)- β -farnesene.

2.4.5 Other Semiochemicals

Cis-3-hexanol was supplied by International Flavours and Fragrances, Haversham UK. L-tryptophan, β -caryophyllene, and β -hydroxybutyric acid were obtained from Sigma-Aldrich chemical Company Co. Ltd, Gillingham, UK.

Chapter 3

Predators selected for the study

Chapter 3 The predators selected for the study

3.1 Carabidae

Ground beetles (Coleoptera: Carabidae) have a very wide distribution being found in all terrestrial habitats from the seashore to the snowline of the highest mountains (Forsythe, 1987). There are some 40,000 species world-wide (Theile, 1977), with nearly 3,000 species found in Europe (Turin, 1981). Carabids have been considered as important predators in agricultural systems since the earliest studies (Forbes, 1883; Balduf, 1935). In addition, some species of carabids are considered to be spermophagous and may play an important role in the control of weeds in arable fields (Brandmayr, 1983). The basic species composition of carabid communities in European agricultural fields is fairly uniform, with about 75 species that are characteristic for agricultural habitats (Heydemann, 1983). Generally however, only five or six species are dominant in a particular field, because of prevailing environmental conditions, making up to 90% of the total number of carabid individuals (Körner, 1990). The genera *Carabus*, *Pterostichus*, *Nebria*, *Harpalus*, *Agonum*, *Calathus*, *Amara*, *Bembidion*, *Clivina*, *Trechus*, *Brachinus* and *Dyschirius* are generally characteristic of agricultural habitats in the Northern temperate zone (Ekschmitt & Wolters, 1997). Largest numbers of carabids are found in cereal crops, followed by root crops, with natural areas in contrast generally less colonised (Dunger, 1983). The number of carabids in arable fields can range from 5 to 50 individuals per square metre.

Carabid species generally fall into one of two types of reproductive strategy (Larson, 1939; Penney, 1966); spring breeders, which overwinter as adults and reproduce in the spring and early summer (Forsythe, 1987), and autumn breeders, which reproduce in the late summer and early autumn and spend the winter as larvae. This breeding cycle can be further divided into 7 sub-categories according to Paarman (1979):

- Spring breeders without the need for long day length for maturation.
- Spring breeders which need long day length for maturation
- Autumn breeders without summer gonad dormancy
- Autumn breeders with summer gonad dormancy
- Autumn breeders with aestivation dormancy
- Species with a 2 year development to adult, with day length controlled dormancy in females
- Species with a 2 year development to adult, with temperature controlled dormancy in females.

In temperate zones species generally only have one generation a year (Ekschmit and Wolters, 1997).

Ground beetles usually lay eggs singly, and the number of eggs laid in a single reproductive period varies according to species. This may be an adaptation to habitat stability, with species living in unstable habitats producing large numbers of eggs while those from stable habitats producing fewer (Forsythe, 1987). Most eggs are deposited into soil, although some species may enclose the eggs in substrate particles for extra protection (Luff, 1987).

The eggs of some *Agonum* species have been found on low lying vegetation (Dicker, 1951). Individual fecundities tend to be much lower in these species, as larval mortality is much lower (Brandmayer, 1983).

The majority of ground beetle species have three larval instars, except *Brachinus* which have five and *Harpalus* and *Amara* which have two (Forsythe, 1987). The biology of the larval stages is not well understood, especially that of the predatory species (Forsythe 1987). Predatory larval feeding habits have been described in some *Pterostichini* (Luff, 1974), and in other species where the adults are predominately phytophagous including *Amara* and *Harpalus* (Forsythe, 1987). However, the role of larvae in the useful predation of agricultural pests is not known (Luff, 1987).

Carabids usually exhibit a diel activity pattern with most of the common temperate field species being nocturnal (Luff, 1978). Whilst visual cues particularly in relation to prey movement seem to be most important in foraging for the few diurnal species, in the nocturnal species, olfactory and tactile cues play a larger role (Griffiths, Wratten and Vickerman, 1985).

Field carabids are active over a wide range of conditions (Eckshmitt & Wolters, 1997). Different species of carabids vary greatly with respect to preferred humidity ranges. Field carabids are evenly distributed over the range of humidity requirements, and the majority are resistant to flooding in general, particularly the larvae which breathe through the exoskeleton and are not enveloped by films of air under water, so moving water can provide oxygen (Thiele, 1977).

Temperature is important in activity cycles, and carabids can be active in the field between +5°C to +50°C (Theile, 1977), however, laboratory experiments, show

preferred temperature ranges to be +10°C to +30°C (Thiele, 1977). Out of 17 field species tested, 16 preferred warm conditions, or were eurythermic (Thiele, 1977). Using pitfall trap catches as a measure of activity, studies have shown that activity is increased by 6.3% for every 1°C increase in temperature (Honêk, 1997). The temperature requirements of different developmental stages vary widely, with high temperatures being particularly important for speedy larval development (Paarman, 1966).

Hedgerows and field margins provide valuable shelter for overwintering carabids, however this effect depends on the composition of the vegetation of the field boundary. Thickly wooded strips 20m wide in Germany were found to be impoverished in field species (Thiele, 1964). However, thinner hedges found in Britain and Poland and other parts of Europe were found to support more field species (Bonkowska, 1970, Coombes and Sotherton, 1986). Field boundaries have been shown to support overwintering carabid populations of up to 900 individuals/m² (Anderson, 1997). Four fields and their boundaries were monitored over four years in Norway, and for every species (except *Clivina fossor* Linneaus) there were found to be more overwintering individuals in the field boundaries than in the fields themselves (Anderson, 1997). Up to 50% of the population from a given field may transfer to neighbouring fields as the result of using field margins as overwintering habitat (Jepson, 1984).

Beetles tend to disperse mainly by running and flying. However, most species which readily carry out flight are small to medium species, and although larger species are capable of flight there are very few flight records for larger species (Luff, 1987). The

distances achieved by flying carabids are unknown, however, a large forest species *Calosoma sycophanta* Linnaeus is known to occasionally fly the English Channel, so there is potential for long distance aerial dispersal of carabids (Luff, 1987). Rates of dispersal over the soil surface have been assessed using mark-recapture experiments, and have been calculated at 5-20m per day, however, low probability of recapturing marked beetles at large distances from the point of release probably leads to an underestimation of the distances travelled (Rivard, 1964; Niesing & Weber 1981; Gordon & McKinlay 1986).

Distribution of beetles within fields is seldom random or even (Luff, 1987). This may be due to a combination of variation in beetle behaviour (Luff, 1986) and in underlying population densities (Luff, 1987). Studies have shown that local altitude and organic matter content of the soil determined much of the within field variation of carabid beetle numbers (Henegveld, 1979). Variation in within field distribution may also be a result of prey aggregations (Calkins & Kirk, 1969; Bryan & Wratten, 1984). However, some carabid species also occur in aggregations when inactive (Forsythe, 1987).

Cultivation and management techniques can have a significant effect on carabid populations in the field. Intercropping can lead to a greater species diversity than that found in monocultures, probably due to greater cover by vegetation (Cárcamo & Spence, 1994). Pesticides, and in particular herbicides, have been shown to reduce both species diversity and numbers of individual carabids by direct toxicity, and by reducing prey and vegetation numbers (Hawthorne and Hassal, 1994). Tillage of

fields has also been shown to reduce beetle populations within fields (Symondson & Liddel, 1996).

3.1.1 *Pterostichus melanarius* Illiger

P. melanarius (Plate 3.1) is the most commonly recorded carabid species throughout northern England and Scotland although in other areas *P. madidus* is found in greater numbers (Symondson & Liddel, 1996). *P. melanarius* is an autumn breeder without summer gonad dormancy (Class three, Paarman, 1979). *P. melanarius* generally overwinters as 3rd instar larvae in the soil, and under rocks, particularly in sheltered areas such as hedgerows and field boundaries. However a few individuals are known to overwinter as adults, so these can emerge and begin to feed and breed quickly when conditions are favourable (Fadl, Purvis & Towey, 1996).

The larvae emerge from overwintering sites during the final half of April and the first half of May, and quickly pupate. The adults emerge in the middle of May and begin to feed in order to mature their sexual organs. Unlike other insects, *P. melanarius* does not need a pollen or nectar source for maturation feeding and obtains all of the resources it requires from its prey (Fadl, Purvis & Towey 1996). The rate of food intake in *P. melanarius* can be extremely high, in excess of three times the body weight of the adult beetle each day (Luff, 1987). *P. melanarius* is a polyphagous predator, and preys on aphids, slug eggs and slugs (Symondson & Liddel 1996, 1996), dipteran eggs and larvae (Finch & Elliot, 1992). This polyphagous habit indicates that *P. melanarius* may be a suitable biological control agent to control a variety of arable pest species.

The beetles are extremely active after pupal emergence, foraging for food and locating a mate, so pitfall trap catches of beetles steadily increase in number from April until the beginning of July. After this time during the main reproductive phase of July to September, the number of adult beetles caught in pitfall traps steadily declines. This is mainly due to the fact that females with mature ovaries are much less active than after first emergence where food was required for maturation (Fadl, Purvis & Towey, 1996). The high levels of mobility during a relatively short period just after pupation, may also be an adaptation for rapid dispersal to ensure survival in a heterogeneous landscape (Den Boer, 1995). *P. melanarius* has been shown to disperse out of fields and into surrounding habitat during this reproduction period (Luff, 1987).

P. melanarius were selected for use in this study because of their high abundance throughout agricultural systems, as their presence early in the season may enable the control of pest numbers before populations can become established, and lead to a greater reduction in pest numbers throughout the entire season. As a polyphagous predator, *P. melanarius* also may prove to be an effective control for several economically important pest species, and be able to survive periods of low pest numbers by switching to alternative food sources.

3.1.2 *Nebria brevicollis* Fabricius

This is the second most commonly recorded carabid species throughout Britain (Forsythe, 1987, Plate 3.2). This is an autumn breeder with gonad development dormancy (Class 4, Paarman, 1977). Larvae emerge in early spring, and quickly

develop into adults (Nelemons, 1987). The adults are only active for 2-3 weeks in early summer during which time they feed rapidly and build up vast fat reserves, before entering a period of diapause (Penny 1966). This period may be triggered by low humidity, high temperatures or long day length (Forsythe, 1987). At the beginning of diapause, the beetles aggregate in groups of up to 80 individuals under stones and logs (Forsythe, 1987). Aggregation declines at the beginning of the breeding season, and is thought to be due to the reduction in the amount of sex pheromone produced which stimulates aggregation before the diapause period (Thiele, 1977). Aggregation and diapause reduces water loss, and enables the beetles to survive periods of low prey numbers, keeps the sexes together in non-breeding periods and enables the sexual organs to develop and mature (Forsythe, 1987). During this period the beetles do not feed and live by oxidation of the fat reserves (Forsythe, 1987). The onset of diapause is controlled by body fat levels, and diapause can be delayed or even prevented by starving the beetles (Forsythe, 1987).

Shorter day length triggers maturation of the gonads, and the ovaries pass through three stages; immature where the ovary is too small to see, developing where the ovaries are small but the ovarioles can begin to be seen, and mature where the ovary has reached full size and contains eggs (Penny, 1966). Eggs are laid between mid October to the beginning of April, with an initial peak of synchronised egg laying followed by a reduced level of oviposition throughout the winter (Luff, 1975). Laboratory reared females laid on average 495 eggs during the season, whereas females reared under field conditions produced an average of 338 eggs each

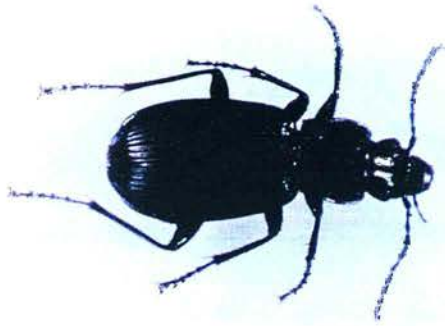


Plate 3.1 *Pterostichus melanarius* Illeger



Plate 3.2 *Nebria brevicollis* Fabricius

(Nelemens, 1987). Viability of the eggs declines as the reproductive season progresses, until after approximately 2.5 months egg viability was only 50% (Nelemens, 1987). First instar larvae development time varied from 12 to 57 days depending on food quality and temperature (with 12°C being the optimum), 2nd instar development at 12°C was approximately 13 days, while 3rd instar development was 71 days at the optimum temperature of 12°C (Nelemens, 1987).

The larval mouthparts of *N. brevicollis* are specialised for catching and eating collembollans (Nelemens, 1987). This enables the larvae to survive the winter when other prey species are unavailable.

N. brevicollis was selected for the study because of its relative abundance throughout the arable field system. They are also polyphagous and may take a variety of pest species, as well as surviving in arable fields in times of prey shortage by switching to an alternative food source. The two main peaks of activity of *N. brevicollis* make it suitable to control early occurring pest species before populations become established, and late occurring pest populations in autumn planted crops before the pests overwinter.

3.2 The hoverfly *Episyrphus balteatus* Degear (Diptera: Syrphidae)

There are over 250 species of hoverfly in Britain, approximately 100 having predaceous larvae, and these belong to the subfamily Syrphinae (Rotheray, 1989). Hoverfly larvae can be divided into two main categories, predaceous larvae, and the rest (Gilbert, 1993). Predaceous hoverfly larvae are unique amongst the dipterans in that they are brightly coloured, instead of the usual dun or white maggots of other

flies. These colours are produced by blood pigments and fat deposits under the skin, and serve to provide camouflage for the larvae, with some species even mimicking bird droppings. This also provides a method of identification of the larvae (Rotheray, 1989).

Amongst hoverfly larvae, a great range of feeding guilds are present, broader than any other insect group, from detritivores, to obligate herbivores and carnivores through to generalist omnivores, with the most important group to man being the predaceous species which feed on aphids (Gilbert, 1993). The larvae of *E. balteatus* are obligate carnivores and prey exclusively on aphids. However, although *E. balteatus* (Plate 3.3) is monophagous it will feed on an extremely wide variety of aphid species (Gilbert, 1993).

Each hoverfly larva will take up to 1200 aphids during its lifetime, *E. balteatus* being one of the most efficient predators in the group, attacking aphids at a faster rate and moving and searching more rapidly than most of the other hoverfly larvae (Gilbert 1993). However, *E. balteatus* also needs more aphids to complete its development (Gilbert 1993). Aphid colonies are frequently decimated by the feeding of hoverfly larvae, resulting in periods of starvation. Starved larvae try to compensate for their nutritional deficiencies by increasing their speed, initiating more rigorous searching behaviours, and ingesting 50% more of the aphids fluids before the aphid husk is



Plate 3.3 *Episyrphus balteatus* Degear (Pierre Watkin, Agents Polliniques,
France)

discarded (Leir & Barlow, 1982). Even with these compensatory mechanisms, starvation still leads to decreased size in the adults, and a reduced fecundity in the females, resulting in less larvae and therefore more food for each individual larva (Cornelius & Barlow, 1980).

All hoverfly larvae have three larval instars, however, the length of the larval stage differs from species to species depending on a combination of factors, including the timing and length of the seasonal appearance of the adults, the occurrence or absence of diapause, and the overwintering stage of the species (Gilbert, 1993). *E. balteatus* has one of the shortest generation times of the group, as its entire reproductive system is geared to produce as many generations in the year as possible. The larval stage can last as little as 10 days, however, this depends on prevailing environmental conditions such as temperature, day-length, humidity and food availability, with less than optimal conditions extending the developmental period (Gilbert, 1993). Even with this very short developmental time there is very little evidence that even in the best years *E. balteatus* can produce more than three generations in the year, and in bad years this may fall to a single annual generation (Gilbert, 1993).

When fully mature the larva empties its digestive system by excreting a black oily liquid, then searches for a suitable place to pupate, usually on the underside of the leaves of the plant that it has been inhabiting (Gilbert, 1993). The larval skin of the third instar is not shed, but instead hardens to form the pupal case, therefore pupation takes place within the last larval skin. Strictly speaking in true pupae in other insects the pupal case forms under the last larval skin which then splits and is cast off. In

hoverflies this stage is called the puparium to distinguish it from the true pupae of other insects (Gilbert, 1993). Pupation lasts from 10 to 14 days (Gilbert, 1993).

Emergence of the adults from the puparium usually takes place early in the day. This is because a newly emerged adult is very vulnerable to predation, and the early emergence allows time for the soft cuticle to harden and for the wings to unfold and dry, before the majority of predators are active (Gilbert, 1993). Males also tend to be smaller and complete development slightly faster than the females. Therefore males generally emerge a few days earlier than the females. This enable the males to feed and mature their reproductive systems before the females emerge, increasing the opportunities to find and mate with as many females as possible (Thornhill & Alcock, 1983).

The majority of *E. balteatus* overwinter as third instar larvae, however a few individuals overwinter as adult gravid females (Gilbert, 1993). This enables adults to appear during the first warm days of spring and to take advantage of the early flowering shrubs and herbs to mature their eggs and get the population off to an early start (Gilbert, 1993). *E. balteatus* are migratory and the majority of British born adults leave in the autumn and cross the English Channel to continental Europe, where favourable conditions may last longer, leaving only a few adults and larvae to overwinter in Britain. There is a corresponding movement from Europe back to Britain in the following spring (Gater & Schmid, 1990).

E. balteatus, like all hoverflies, are excellent flyers. The wing in cross section is not flat but is shaped like an aircraft wing, and is also corrugated and covered with

microscopic hairs to improve its aerodynamic properties. The large thoracic muscles which control the wings comprise about 15% of total body weight of the insect and can produce up to 250 wingbeats per second. In hovering the wings move in a flat and oblique figure of eight, bending and twisting on the stroke, and so providing plenty of lift. These combine to give hoverflies aerial mastery that is only rivalled by that of the hummingbirds (Gilbert, 1993).

Adult hoverflies are diurnal, but have the extremely large eyes usually only associated with nocturnal insects. This is again an adaptation to flight, as the hoverflies are such fast flyers they need very accurate vision to be able to control their movements. Unlike in other flies, in flight the head is also perfectly still in relation to the thorax, providing better vision during flight. However the good vision resulting from these adaptations also provides advantages in searching for oviposition sites near to larval prey (Gilbert, 1993).

Adult females emerge from the puparium with completely undeveloped eggs, males also emerge with unmaturing reproductive systems, particularly the accessory glands, so maturation feeding is extremely important (Gilbert 1993). Pollen grains are a rich source of essential proteins and amino acids necessary for maturation, and contain about 25% by weight of energy rich carbohydrates and lipids. On first emergence the insects feed almost exclusively on pollen (Gilbert, 1993). However the energy present in pollen is more difficult to metabolise than simple sugars, and therefore can be made available only slowly (Gilbert 1993). Nectar is almost a completely saturated sugar solution, with very few amino acids and proteins so is not good for maturation feeding, however it makes for a readily metabolisable fuel source that

satisfies the large energy demand needed for flight (Gilbert, 1993). Consequently mature adult males feed almost exclusively on nectar, as they carry out the majority of the flight behaviour during mate location. Females constantly need pollen for the maturation of subsequent batches of eggs but also need a limited supply of energy for flight, so mature adult females feed on a mixture of pollen and nectar (Gilbert, 1993). *E. balteatus* exhibits preference for certain types of flowers (Hickman and Wratten, 1996).

Mating in *E. balteatus* is complex, the females have a greater investment in the offspring in terms of energy and resources, so it is of benefit to the female to choose the best possible male to father the offspring. It is of benefit to the male to fertilise as many females as possible, so mate choice generally falls to the female (Thornhill & Alcock, 1983). Males display by hovering prominently in the sun, near vegetation with the light showing their bright colours to best advantage. They dart and hover from one area to another to patrol for females, and chase any passing insect, returning when unsuccessful (Thornhill and Alcock, 1983). The female has to preserve her energy reserves for egg production, so makes little attempt to seek out males, and is generally courted while resting or feeding, with mating taking only a few minutes. The females storing the sperm in spermathecae, (Thornhill & Alcock, 1983).

Once the eggs are mature, the female searches for a suitable oviposition site, usually in or at the edge of potential larval food (Gilbert, 1993). A variety of cues can be used in searching and oviposition behaviour and these can be ranked in the order that they are carried out (Table 3.1).



Table.3.1, Cues for oviposition in *E. balteatus* (modified from Chandler, 1966).

Sense	Cues
Visual	1. Size of plant patch
	2. Density of plant patch
	3. Colour of plants
	4. Form of plants
Olfactory	5. Green leaf volatiles
	6. Aphid pheromones
Taste	7. Honeydew
Visual	8. Size and position of colony
	9. Shape of aphid
	10. Aphid movement
Tactile	11. Egg size
Female behavioural response	Cues involved
Habitat selection	1,2,3
Plant selection	2,3,4,5
Aphid colony selection	6,7,8,9,10
Egg site selection	8,9,10,11

Olfactory cues play an important role in oviposition site selection; if a female is unable to locate aphid patches, egg maturation still continues, however, the eggs are then reabsorbed into the female, which help extend the female's life (Gilbert, 1993).

Adult *E. balteatus* can live for approximately for 3 weeks, however few die of old age. The vast majority of adults hoverflies are eaten by predators particularly birds (Gilbert, 1993). It was thought that the bright colours of hoverflies were a form of batesian mimicry of bees and wasps, designed to deter predators. Experiments show that in general the bright colours of hoverflies do not generally deter predators, and they deal with hoverflies in the way they would normal flies (Davies, 1977). However the exception to this is *E. balteatus*, whose coloration seems to deter birds even though to human eyes it is not a particularly effective wasp mimic (Dittrich *et al.*, 1993).

E. balteatus was selected for this study because it is a highly abundant in arable fields, is easy to culture and reproduces very quickly under both laboratory and field conditions. The larvae are monophagous preying exclusively on aphids, so a comparison with polyphagous carabids could be carried out. Because of its low generation time and reproductive strategies, *E. balteatus* appears in the field early in the season, before aphid populations have become established, and have the potential to control aphid numbers when populations are low. *E. balteatus* is also an extremely efficient predator also enabling this species to have a large effect on pest populations.

3.3 Ladybirds *Coccinella septempunctata* Linnaeus and *Adalia bipunctata* Linnaeus (*Coleoptera: Coccinellidae*).

There are 475 species of ladybirds from 57 genera throughout the world (Majerus, 1994). In the UK, the first ladybird eggs begin to appear on vegetation around late May/ early June just after the emergence of the first adults from overwintering. On hatching the larvae eat the egg case to provide its first energy requirements (Majerus, 1994). After eating their egg case, early hatching larvae may eat unhatched eggs from the same clutch. This is because the dispersal phase away from the egg is one of the most vulnerable periods for the larvae, it has to locate food resources quickly or face starvation, consequently cannibalism of eggs provides extra energy for the foraging larvae (Majerus, 1994). However, the female generally oviposits in the vicinity of prey colonies, so the larvae usually find prey nearby. Both the 7 spot ladybird, *Coccinella septempunctata* (Plate 3.4), and the 2 spot ladybird, *Adalia bipunctata* (Plate 3.5), are generalist predators. Both the adults and larvae are carnivorous and both stages will prey on aphids as their primary food source, however, they will also take a range of secondary food items, such as mites, honeydew, pollen, nectar, mildews, adelgids, and other Coccinellidae.

The larvae pass through four larval instars before the final moult and pupation. The length of the larval periods varies according to prevailing climatic conditions such as temperature, humidity and day length. However, in both species, the larvae generally take three to four weeks to complete development (Majerus, 1994). When fully developed, the larvae cease feeding and start to pupate. Choice of pupation site does

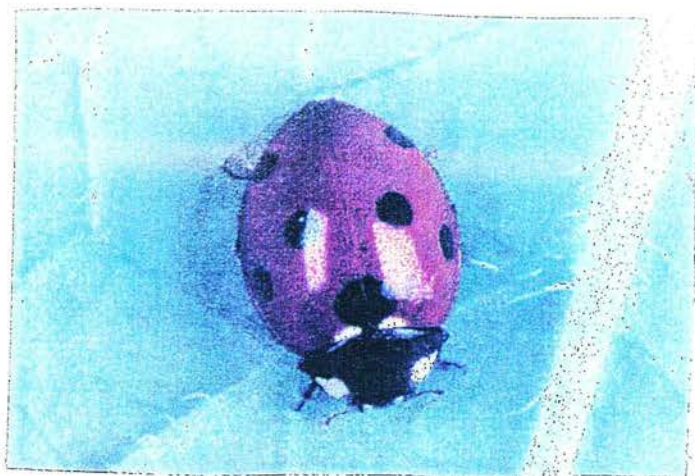


Plate 3.4 *Coccinella septempunctata* Linneaus



Plate 3.5 *Adalia bipunctata* Linneaus (Majerus, 1994).

not appear to be particularly important, and the larvae appear to pupate wherever they are at the appropriate time (Majerus, 1994). The larvae attaches itself to the substrate by its rear end and hunches into the pre-pupa position. After 24 to 48 hours after it first becomes immobile, the skin of the last larval instar splits transversely and longitudinally and the pupa wriggles to shed the skin. The pupa is at first cream coloured, but darkens to black as the pupal case hardens (Majerus, 1994). Approximately 7 days after pupation (depending on temperature) the adult emerges from the pupa. The larva generally pupates with its head pointing downwards, so gravity helps adults to emerge (Majerus, 1994). Generally, the new generation of adults begin to emerge in late June, while the old overwintering generation begin to die. Reproductively exhausted, they succumb to fungal diseases and predators such as ground beetles (Majerus, 1994).

On first emergence, the adults are white or pale yellow, and cling onto the pupal case, while their elytra harden, usually reaching full adult colouration within 24 hours, although the colour continually deepens as ladybirds age (Majerus, 1994).

After emergence, young adults begin maturation feeding. Adult *A. bipunctata* can reach full sexual maturity within a week, however, it takes longer in *C. septempunctata*. For *A. bipunctata* there is some potential for a limited amount of cross breeding between the generations, particularly if there are favourable climatic conditions in late spring/ early summer. In 2 spot ladybirds older individuals of both sexes may mate with younger individuals, however, matings between young males and old females are much more common, because male longevity is slightly less than that of females, and young females are less reproductively active than males. In *C.*

septempunctata, females rarely mate before overwintering, so young males mate with old females, however, there are fewer cross generation matings in *C. septempunctata* because of the length of time it takes to reach sexual maturation. Cross generation mating only produces a partial second generation each year, but a single generation is more usual (Majerus, 1994).

Throughout the summer, ladybirds feed on aphids and other items to build up fat reserves in preparation for the winter. As the summer progresses, natural changes in plant chemistry trigger the production of the sexual winged phase of the aphid lifecycle which leads to dispersal from the aphids summer hosts. During this period, ladybirds will also disperse, sometimes in large numbers, to seek for food elsewhere. During this period, high temperatures increase ladybird metabolic rate, and this, coupled with increased movement by the ladybirds leads to energy reserves being used more quickly, so more prey must be found if the ladybird is to survive the winter (Majerus, 1994).

Shortening days and deteriorating vegetation leads to prey becoming increasingly more difficult to find, so most ladybirds give up searching for prey and begin to look for appropriate overwintering sites. *A. bipunctata* is one of the first species to move to overwintering sites, but this is closely followed by *C. septempunctata* as aphid population numbers quickly fall (Majerus, 1994). *C. septempunctata* is not particularly selective when it come to overwintering sites, and will use almost any sheltered spot; amongst conifer foliage, in curled dead leaves, hollow plant stems, grass tussocks, or in leaf rosettes. In the shelter of woodlands they may also overwinter on exposed trunks, branches and low vegetation. They also aggregate

together in mixed sex groups of up to a few dozen adults to overwinter (Majerus, 1994). *A. bipunctata* is more selective with natural overwintering sites traditionally including the undersides of bark, in cracks or any other sheltered position on tree trunks, but invariably several feet above the ground. However, it is now more common to find them overwintering in sheds, outbuildings and even houses if the room is cold, where they can be found in groups of up to several hundred individuals. The methods of this aggregation are not well understood, but are thought to involve use of a pheromone (Majerus, 1994). The same overwintering sites also tend to be used year after year, even though next years overwintering adults are a separate generation, so some form of pheromone may be laid down at the site by the previous year's generation (Majerus, 1994).

Mortality of ladybirds as they overwinter is generally very high, and depends on the levels of reserves the ladybirds were able to accumulate during the summer. However, ladybirds are extremely hardy and *C. septempunctata* have been found frozen in blocks of ice, only to emerge safely in the spring. *C. septempunctata* has also been found to emerge on sunny days during the winter to bask in the sun, returning to the overwintering site before nightfall (Majerus, 1994).

Adults begin to emerge from overwintering sites in the second half of April, when food becomes available. However, the cues which bring the ladybirds to full activity after the winter are unknown, but they probably involve increasing temperature and day length (Majerus, 1994).

After feeding, ladybirds begin to look for mates, however unlike in other insect species, this does not seem to involve long range attraction to volatile sex

pheromones; rather, males seem to find females by bumping into them more or less at random, although there are probably some short range visual stimuli involved. The male clambers onto the back of any other ladybird it encounters, however, if the other ladybird is also male the encounter is soon broken, but it is unknown if this is through the action of some male specific contact cue, or if it is simply the lack of a female specific cue (Majerus, 1994). In *A. bipunctata* females have recently been shown to produce a cue that excites the male when it contacts the elytra (Hemptine & Dixon, 1997).

Females demonstrate a very strong rejection behaviour when mounted by a male from a different species; this is probably due to the lack of a species specific recognition cue (Majerus 1994). If females of the same species are located, mating is not assured as the female will reject the male if she is hungry, has recently mated, or is about to lay eggs (Majerus, 1994). In *A. bipunctata*, some females carry genetic information that leads them to prefer mates with specific characteristics, and reject those that do not carry these (Majerus, 1994). Mating takes between 1 to 8 hours, with many species being very promiscuous. *A. bipunctata* needs repeated matings to maintain a high level of egg fertility, and a female will mate on average 20 times during the spring reproductive period. Promiscuity also helps to reduce the levels of inbreeding in the population. The eggs are laid in batches of up to 20 on vegetation, usually in the vicinity of prey species.

Ladybirds were selected for the study because of their relative abundance throughout arable systems, their general efficiency as predators.

3.4 Provision of insect material

3.4.1 Aphids *Myzus persicae* and *Sitobion avenae*

Myzus persicae were maintained on Chinese cabbage plants, *Brassica chinensis* (var. China Pride) (Chiltern Seeds, Ulverston, UK). Plastic seed trays (20 x 30 cm) were filled with compost (John Innes Irish moss peat). The seed was spread over the surface of the compost so that one packet (2g) provided enough seeds for 2 trays. The seed trays were placed in a 1mm nylon mesh covered wooden framed cage (1.8m x 1m x 0.7m) in a glasshouse. The mesh was fine enough to prevent any insects gaining access to the plants, or leaving the cage.

The seeds were watered 3 times weekly and left to grow and germinate for approximately 3 weeks at which time they were transferred to individual 15cm diameter plastic plant pots filled with John Innes Irish moss peat.

Myzus persicae (CSL, York) were placed on the Chinese cabbage plants which and placed in a 1mm nylon mesh covered cage (30 x 30 x 30cm) in an insectary. They were maintained at $20\pm 2^{\circ}\text{C}$ with a 16:8 hour light dark regime. The plants were watered 3 times per week and fresh plants were added as necessary.

Sitobion avenae (CSL, York) were maintained on winter wheat cv. Riband. Plastic seed (10 x 20 cm) trays were filled with John Innes Irish moss peat. Wheat seeds were planted in the peat as 4 rows of 4 seeds, the rows being set approximately 3cm apart. The trays were watered and placed in a cage (1.8 x 1 x 0.7m) in a glass house and maintained at $20\pm 2^{\circ}\text{C}$ and a 16: 8 hour light: dark regime.

When the seedlings reached the 2 leaf stage they were moved to a 1mm nylon mesh covered cage (30 x 30 x 30 cm) in the insectary, and *Sitobion avenae* nymphs placed on the plants. They were maintained at $20\pm 2^{\circ}\text{C}$ with a 16:8 hour light dark regime. The plants were watered 3 times per week and fresh plants added as necessary.

3.4.2 Carabidae *Pterostichus melanarius* and *Nebria brevicollis*

Live *P. melanarius* and *N. brevicollis* were captured using dry pitfall traps with a cover that prevented access to the trap by small mammals but allowed carabids to enter. The traps were placed at 1m intervals in setaside land at Boghall Farm, Midlothian (UK), and were emptied once per week throughout the summer. The beetles were placed in a plastic container with a hole in the lid covered in 1mm nylon mesh that allowed ventilation into the containers but prevented the beetles from escaping. To reduce the incidence of cannibalism in the container the beetles were transported as quickly as possible back to the laboratory.

The beetles were maintained in a plastic seed tray (30 x 20cm) covered with a perspex incubator. The bottom of the seed tray was covered in approximately 5cm depth of soil obtained from a winter wheat field in which natural weed seeds present in the soil had been allowed to germinate to provide cover for the beetles. The incubator was kept in a plant growth cabinet (Fisons Ltd) which was set to provide a 18:6 hour light: dark regime, a temperature of $10\pm 1^{\circ}\text{C}$, and 80% relative humidity. Once a week the beetles were provided with a petri dish containing cat food (Whiskas Cat Foods, Melton Mowbray, UK). Provided that the beetles were well fed, cannibalism was not a significant problem within the seed tray.

The carabids were sexed according to the method of Haliday (1977). The beetles were lightly anaesthetised using CO₂; under the anaesthetic the beetles extended their sexual organs between the last segment of the abdomen and the elytra. The anaesthetised beetles were examined under a dissecting microscope and the sex of the beetles determined.

3.4.3 *Episyrphus balteatus* culture

Episyrphus balteatus were reared in the laboratory according to the method of Frazer (1972). Adult *E. balteatus* were captured using a sweep net and pooter from vegetation at the field margins of setaside land at Boghall Farm, Midlothian, UK. The captured insects were placed in a plastic container with a perforated lid in which a few flowers from the surrounding vegetation had been placed. The insects were transported to the laboratory as quickly as possible to avoid stressing them.

The insects were maintained at $20 \pm 2^{\circ}\text{C}$ with a 16:8 hour light: dark regime. The adults were kept in wooden framed cages (30cm x 30cm x 30cm) covered in a 1mm nylon mesh, and provided with cotton wool soaked in a 10% sucrose solution and a small petri dish containing finely ground bee pollen granules (Rowse Honey LTD, UK) to provide a protein source for oogenesis. The cotton wool and pollen were replaced 3 times weekly.

Chinese cabbage plants *Brassica chinensis* (cv. China Pride) infested with *Myzus persicae*, were placed in the adult cage for 4 hours every 3 days, to provide a surface for oviposition and to ensure that all the eggs were approximately the same age, so that larvae would develop at approximately the same rate. The cabbage plants were

then removed to a separate larval cage and provided with fresh aphids until larval pupation occurred. Once the larvae had pupated, pupae were collected by hand and placed in a plastic petri-dish, which was placed in the adult cage until adult emergence.

The adults were sexed according to the method of Frazer (1972). In females, both the eyes meet at the top of the head and the final segment of the abdomen tapers to a point. In males, there is a gap between the eyes at the top of the head and the final segment of the abdomen is rounded.

3.4.4 Coccinellids *Coccinella septempunctata* and *Adalia bipunctata*.

Egg batches of *Coccinella septempunctata*, the seven spot ladybird and *A. bipunctata*, the two spot ladybird were obtained from M. Majerus, Department of Zoology, Cambridge University, UK. The eggs were kept in ventilated petri dishes and upon hatching were provided with a surplus of the aphid *M. persicae* for food. The larval dishes were maintained in an insectary at $20\pm 2^{\circ}\text{C}$ and a 16:8 hour light dark regime. Since the older larvae were cannibalistic it was necessary to place the third instar larvae in separate 5cm diameter petri dishes.

The adults were released into a wooden framed 1mm nylon mesh covered cage (30 x 30 x 30cm), under the same ambient conditions as above, but with light intensity in excess of 1000 lux, otherwise the adults would not breed. The adults were provided with Chinese cabbage plants (cv. China pride) infested with *M. persicae*, cotton wool soaked in 10% sucrose solution and crushed bee pollen granules. Fresh pollen and sucrose were placed in the cage 3 times per week. The plants were checked for eggs

3 times per week. The section of the leaf containing eggs was removed and placed in a larval petri dish.

The coccinellidae were then sexed according to the method of Haliday (1977). The beetles were lightly anaesthetised using CO₂. Under the anaesthetic, the beetles extended their sexual organs between the last segment of the abdomen and the elytra. The anaesthetised beetles were examined under a dissecting microscope and the sex of the beetles determined.

Chapter 4

Electrophysiological response to semiochemicals

Chapter 4. Electrophysiological response to semiochemicals

4.1 Introduction

Many studies have been carried out on the sense organs used to detect semiochemicals from the prey or prey habitat (Kainoh, 1990). The antennae have been identified as the most important sense organ for long range orientation in insects (Weseloh, 1972; Richardson, 1972; Borden *et al.*, 1978), although other sensilla have been identified on other parts of an insects body such as the tarsi or ovipositor (Greany *et al.*, 1977; Thompson, 1983).

Electroantennograms or EAGs can be carried out to establish the response of an insects antennal sensilla to volatile compounds (Sazanov, 1988). The EAG response is a slow graded summated receptor potential of all the olfactory cells on the insect antenna (Schneider, 1963). This technique has been used to identify the pheromone/ kairomone / synomone relationships of several predator-prey systems (Ramachandron & Norris, 1991; Vet *et al.*, 1990). The electrophysiological recording of insect antennal responses has been refined to enable the recording of responses from a single receptor cell in the insects antennae (Boeckh, 1962). This enables different types of sensilla specific to different chemicals to be identified. (Davis & Sokolov, 1976). However with plant extracts, which are not single chemicals but consist of a complex mixture of volatiles, this technique would be unsuitable. Single cell recordings could only record the antennal response to one of the compounds in the mixture, whereas EAG recordings are a summated response of all the antennal receptors to all of the compounds in the mixture (Evans, 1991).

EAGs have been invaluable in the isolation and identification of attractant compounds (both pheromones and plant volatiles) from natural sources (Evans, 1991). The technique has been used to identify semiochemicals such as common green leaf volatiles which act as cues for phytophagous insects including the Colorado potato beetle *Leptinotarsa decemlineata* Say (Visser *et al.*, 1979), the boll weevil *Anthonomous grandis* Bohemen (Dickens, 1984) and the cabbage seed weevil *Ceutorhyncus assimilis* Payk. (Evans & Allen-Williams, 1989). It has been used to determine the antennal responses of aphidophagous hoverflies (Hood Henderson & Wellington, 1982a), and parasitoids (Vet *et al.*, 1982) to prey and habitat location cues.

Therefore this technique is appropriate for rapid screening of large numbers of semiochemicals, however, it will only give an indication that insect can detect the semiochemical. The behavioural response has to be monitored in a behavioural assay such as an olfactometer or wind tunnel.

4.2 Materials and methods

Insects were collected in the field and cultured in the laboratory (see Chapter 3). Electroantennograms (EAGs) were obtained using a standard method (Evans and Allen-Williams, 1992) as illustrated in Fig. 4.1

The insects were removed from the culture 24 hours before the experiment, lightly anaethetised with CO₂ and sexed according to the method of Frazer (1976) and Haliday (1977). The insects were then placed in individual petri-dishes containing damp filter paper until the experiment.

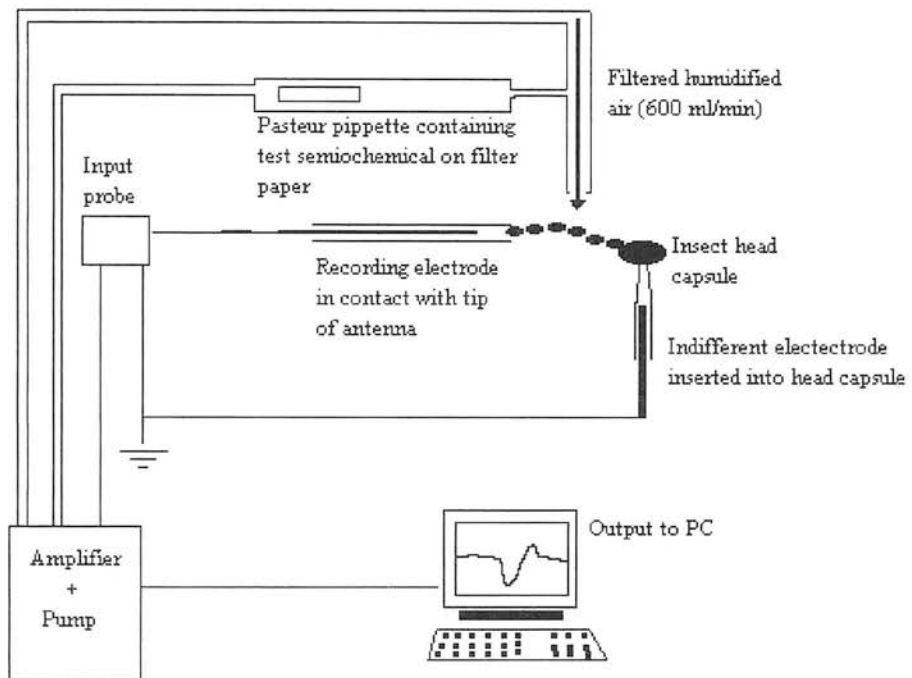


Fig. 4.1 The EAG experimental design (modified from Evans, 1991).

The preparation and air delivery system were both housed in a Faraday cage to minimise electrical noise. The pipette and computer were all located outside the cage.

The head of the insect was removed and mounted on a glass electrode. The glass 1.5mm diameter capillary tubes (Haematocrit tubes, Denly Instruments, Daventry, UK) were pulled using a Sri glass microelectrode puller (Palmer Bioscience LTD, Edinbridge, UK), to obtain a fine tip approximately 10-20 μm in diameter at the end of the electrode. The electrodes were filled with Beadle Ephrussi ring solution (7.5g NaCl, 0.35g KCL, 0.29g $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, in 1L distilled water) as an electrolytic medium. Silver wire (o.d 0.5mm, FSA Laboratory Supplies, Loughborough, Leicestershire) connected the preparation to the recording instruments (Fig.4.1). The electrodes were securely held in two electrode holders on two micromanipulators (Gallenkamp, Loughborough, Leicestershire), which are held steady by their magnetised bases on a metal sheet. One glass electrode (the indifferent electrode) was connected to ground and the other (the recording electrode) to a P-01 universal probe, which in turn was connected to the AM-05 EAG amplifier (Synchem, Hilversum, the Netherlands). The output from the amplifier was connected to an IBM PC running the EAG for Windows software package (Synchem, Hilversum, the Netherlands). The preparation, manipulators, probe, microscope, and light source were all held in an aluminium Faraday cage to ensure there was no external electrical interference.

By manoeuvring the reference electrode tip to touch the tip of the insects antenna (Fig. 4.1) the electrical circuit was completed and the signal from the insect's

antenna displayed on the PC monitor screen. If an adequate signal was obtained from the antenna the filtered air flow over the preparation was adjusted to 600 ml/min, and the end of the metal tube (o.d 1cm diameter) delivering the air was placed 5mm from the preparation. Air filtered through activated charcoal to remove any volatiles was passed over the preparation (Stimulus Controller C5-05/b, Syntech, Hilversum, the Netherlands).

A dilution series of the test semiochemical, or extract was prepared in methanol. The dilution series ran from a 10^{-10} dilution to fully concentrated. Fully concentrated test materials were undiluted *Nepeta* or *Phacelia* extract, materials in liquid form at the same concentration as supplied by the chemical company and a 0.1 mol solution of L-tryptophan test stimuli were used in the order shown in Table 4.1. A 10 μ l aliquot of the test solution was pipetted onto a piece of glass microfibre filter paper (GF/C, Whatman, Maidstone, Kent) and was placed in a 5ml disposable glass Pasteur pipette (John Poulton Ltd, Barking, Essex, UK). The pipette was left for 10 seconds to allow the test semiochemical to evaporate and equilibrate. The pipette was then connected to the air pump using rubber tubing. The tip of the pipette was placed into a hole in the metal air delivery tube. On depression of a foot pedal, a 0.5 second pulse of air at 600 ml/min was diverted down the Pasteur pipette over the preparation. At the same time the antennal response was recorded automatically on the PC, the program being set to record for five seconds before the stimulation and 5 seconds after.

As the antennal responses of a preparation tend to decline throughout the range of tests, the responses (measured as maximum amplitude) to the test compounds were

Table 4.1 **Order of stimuli used in EAG studies.**

1. Air
2. Initial standard stimulus (10^{-2} dilution cis-3-hexanol)
3. Solvent control (methanol)
4. Standard stimulus
5. 10^{-10} dilution test stimulus
6. 10^{-8} dilution test stimulus
7. Standard stimulus
8. 10^{-6} dilution test stimulus
9. 10^{-4} dilution test stimulus
10. Standard stimulus
11. 10^{-2} dilution test stimulus
12. 10^{-1} dilution test stimulus
13. Standard stimulus
14. 0.5 dilution test stimulus
15. Fully concentrated test stimulus
16. Standard stimulus
17. Solvent control (methanol)
18. Standard stimulus
19. Air

Table 4.2 **List of volatile compounds screened for EAG response of *Pterostichus melanarius*, *Nebria brevicollis*, and *Episyrphus balteatus***

No.

Compounds associated with honeydew

- 1 Acetaldehyde
- 2 Acid hydrolysed L-tryptophan
- 3 Artificial aphid honeydew

Green leaf volatiles

- 4 cis-3-hexanol
- 5 β -Caryophyllene
- 6 β -hydroxybutyric acid

Plant extracts

- 7 *Nepeta cataria* whole plant extract
- 8 *Phacelia tanacetifolia* leaf extract
- 9 *Phacelia tanacetifolia* flower extract

Compounds associated with prey pheromones

- 10 (E)- β -farnesene

corrected with reference to a standard compound. The standard compound used in the assays was cis-3-hexanol, commonly found in the green leaf volatile complex of many plants and used as a standard in previous EAG studies (Evans & Allan Williams, 1989). The application of a standard 10^{-2} stimulus (10 μ l/ml of methanol) to the insect antenna usually gave a good response from the preparation. The antenna was treated with the standard compound every 2 stimulations. (Table, 4.1).

The choice of test compounds (Table 4.2) was based on those previously identified from the literature. (E)- β -farnesene was prepared according to the modified method of (Dawson *et al*, 1984, Chapter 2). *Nepeta* and *Phacelia* extracts and artificial honeydew solution were prepared according to the method in outlined in Chapter 2. The rest of the compounds were obtained from commercial suppliers. Other compounds were obtained from Sigma Aldrich Chemical Company Ltd, Gillingham, UK, except for the cis-3-hexanol which was obtained from International Flavours and Fragrances, Haversham, UK.

4.2.1 Analysis of data

For each semiochemical 6 males and 6 females were tested. At this stage the responses were analysed, as 6 insects was judged as the minimum sample size to provide significant results. If there was found to be no difference between responses of males and females, then individuals of both sexes were pooled together giving a sample size of 12. If however, there was a significant difference between the responses of males and females then four more individuals for both males and

females were tested and the responses for each sex analysed separately providing a sample size of 10.

The EAG values (mV) obtained were adjusted to compensate for antennal fatigue. The individual values for the solvent and test stimuli were compared to the values for the standard stimuli using the following formula (Blackwell *et al.*, 1997):

$$C = (V \times 2 / (S_1 + S_2)) \times S_i$$

Where:

C = Corrected EAG value (mV)

V= Test EAG value (mV)

S₁ = Standard stimulus prior to the test stimulus (mV)

S₂ = Standard stimulus following the test stimulus (mV)

S_i = Initial standard stimulus (mV)

For example for female *E. balteatus* the EAG values obtained are shown in Table 4.3.

So the corrected EAG value for fully concentrated *Phacelia* flower extract:-

$$C = (1.704 \times 2 / (6.144 + 4.987)) \times 10.42 = 3.19 \text{ mV.}$$

The mean of the corrected values for the solvent and test stimuli were calculated, and values for the solvent were subtracted from the values for the test stimuli, giving the absolute corrected values in mV for the test stimuli. This represent the depolarisation of the antennal sensilla due only to the test stimuli and not to the solvent.

Table 4.3 EAG values for a female *E. balteatus* to *Phacelia* flower extract

Stimulus	EAG value (mV)
Air	0.004
Initial cis-3-hexanol	10.42
methanol	0.974
cis-3-hexanol	8.103
10^{-10} <i>Phacelia</i>	1.250
10^{-8} <i>Phacelia</i>	0.892
cis-3-hexanol	8.445
10^{-6} <i>Phacelia</i>	0.998
10^{-4} <i>Phacelia</i>	1.082
cis-3-hexanol	7.737
10^{-2} <i>Phacelia</i>	1.256
10^{-1} <i>Phacelia</i>	1.196
cis-3-hexanol	6.144
0.5 <i>Phacelia</i>	1.505
fully concentrated <i>Phacelia</i>	1.704
cis-3-hexanol	4.987
methanol	0.486
cis-3-hexanol	4.576
Air	0.058

The data were analysed by a single factor analysis of variance (ANOVA) (Blackwell, 1997). If the analysis of variance showed a significant difference between responses to the control and semiochemical then the threshold concentration was determined using a Newman-Keuls multiple range test (Zar, 1982).

4.3 Results

4.3.1 *P. melanarius*.

For both male and female *P. melanarius* there was no significant difference between the antennal responses to the methanol control and artificial honeydew ($P > 0.05$, Fig. 4.3), acetaldehyde ($P > 0.05$, Fig. 4.2), β -caryophyllene ($P > 0.05$, Fig. 4.4), β -hydroxybutyric acid ($P > 0.05$, Fig. 4.5), L-tryptophan ($P > 0.05$, Fig. 4.6), *Nepeta cataria* extract ($P > 0.05$, Fig. 4.8) leaf extract and *Phacelia* leaf extract ($P > 0.05$, Fig. 4.7).

Cis-3-hexanol elicited a significant dose-response from both male and female *P. melanarius* ($P < 0.01$, Fig. 4.9), as did *Phacelia* flower extract ($P < 0.05$, Fig. 4.10).

(E)- β -farnesene elicited a significant dose dependent response in male *P. melanarius* ($P < 0.001$, Fig. 4.11), although females did exhibit a slight response to (E)- β -farnesene, it was not significantly different from that obtained to methanol ($P = 0.058$). P values for the EAG responses of *P. melanarius* are shown in Appendix 1, Table 10.1.

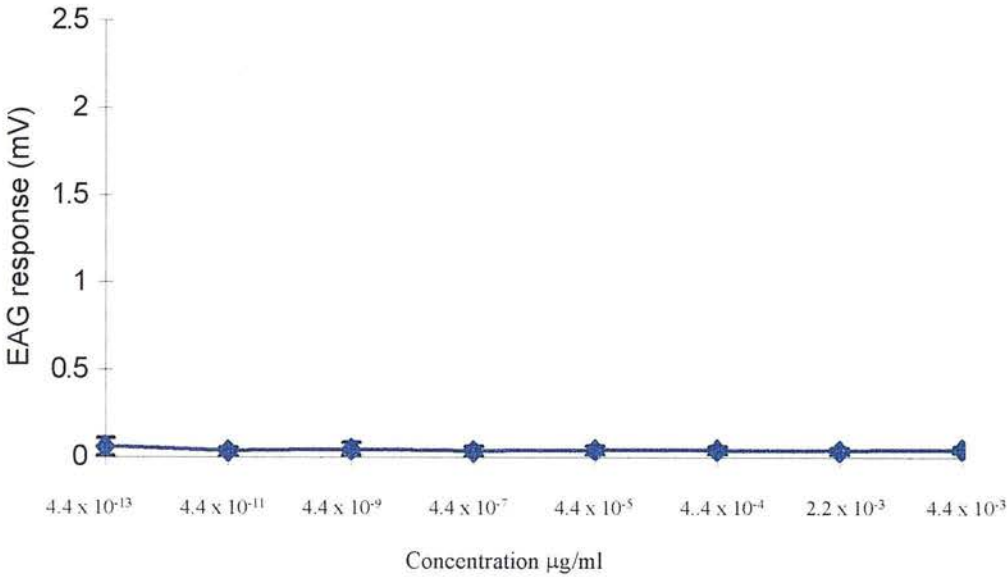


Fig. 4.2 The EAG response in mV (\pm SE) of *P. melanarius* to acetaldehyde (sample size=12)

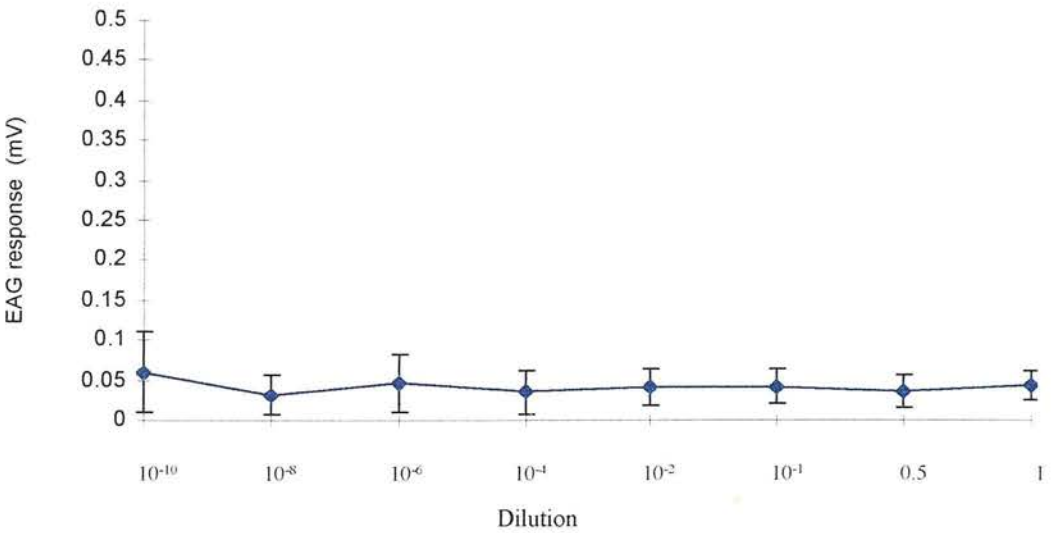


Fig. 4.3 The EAG response in mV (\pm SE) of *P. melanarius* to artificial aphid honeydew (sample size=12)

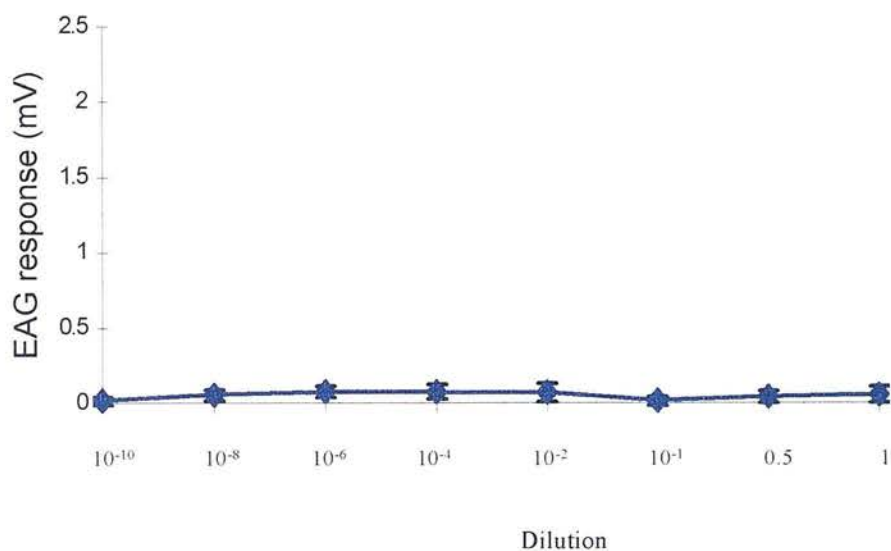


Fig. 4.4 The EAG response in mV (\pm SE) of *P. melanarius* to β -caryophyllene (sample size=12)

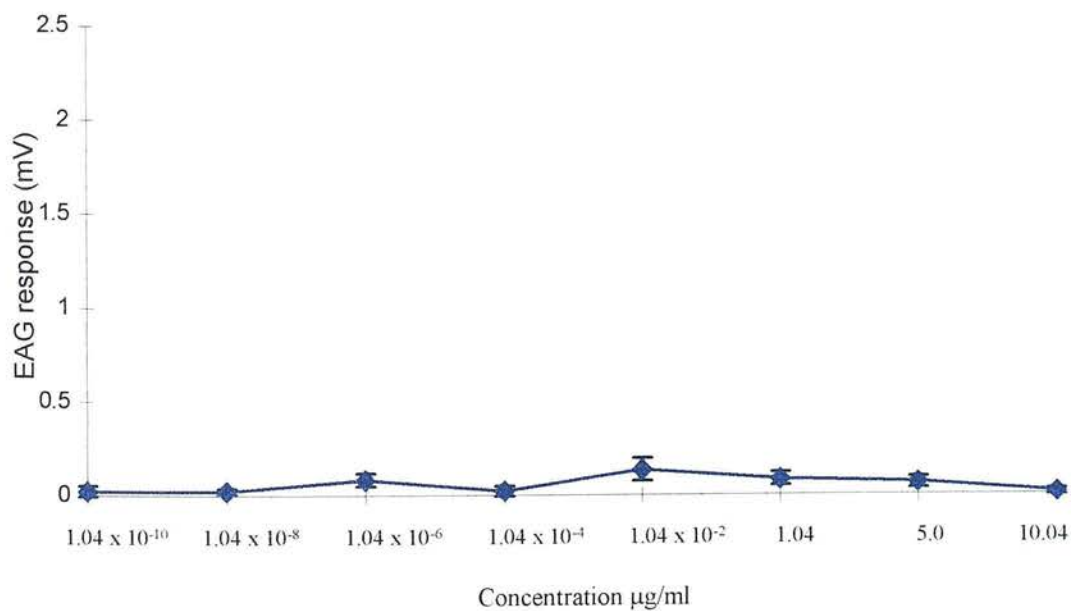
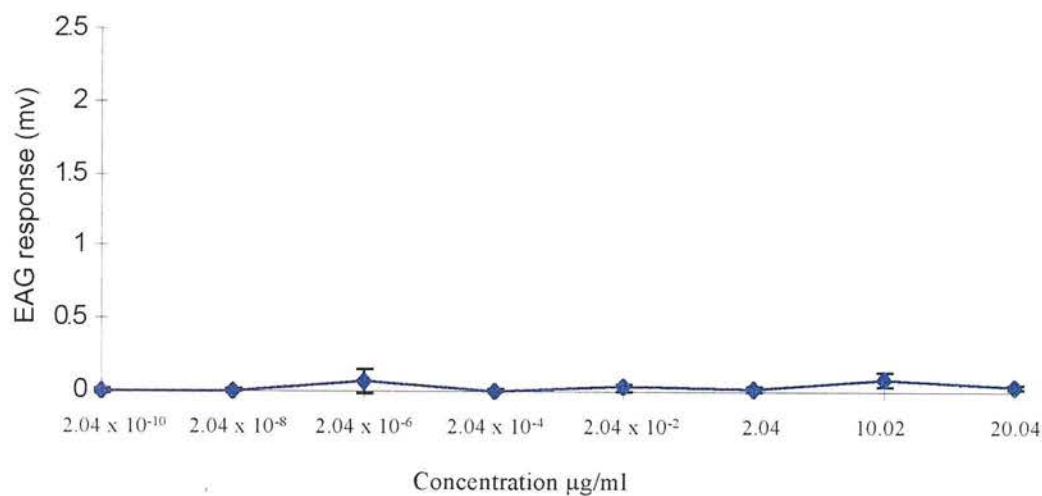


Fig. 4.5 The EAG response in mV (\pm SE) of *P. melanarius* to β -hydroxybutyric acid (sample size=12)



ig. 4.6 The EAG response in mV (\pm SE) of *P. melanarius* to L-tryptophan (sample size=12) F

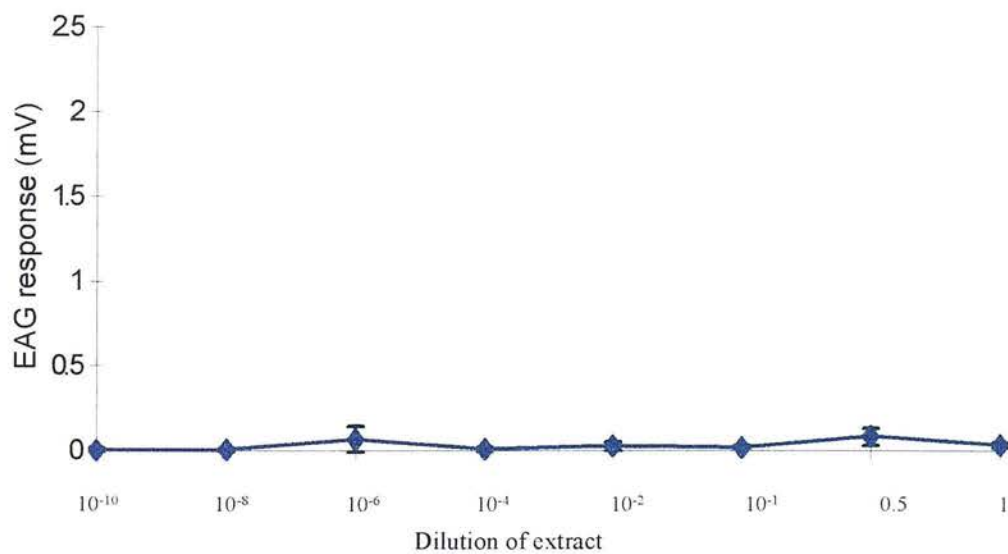


Fig. 4.7 The EAG response in mV (\pm SE) of *P. melanarius* to *Phacelia* leaf extract (sample size=12)

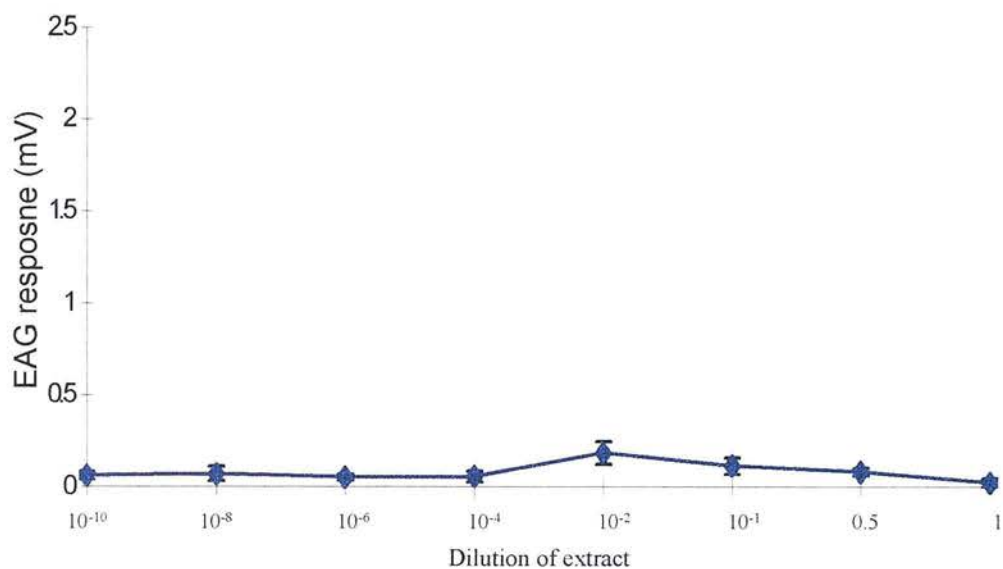


Fig. 4.8 The EAG response in mV (\pm SE) of *P. melanarius* to *Nepeta* extract (sample size=12)

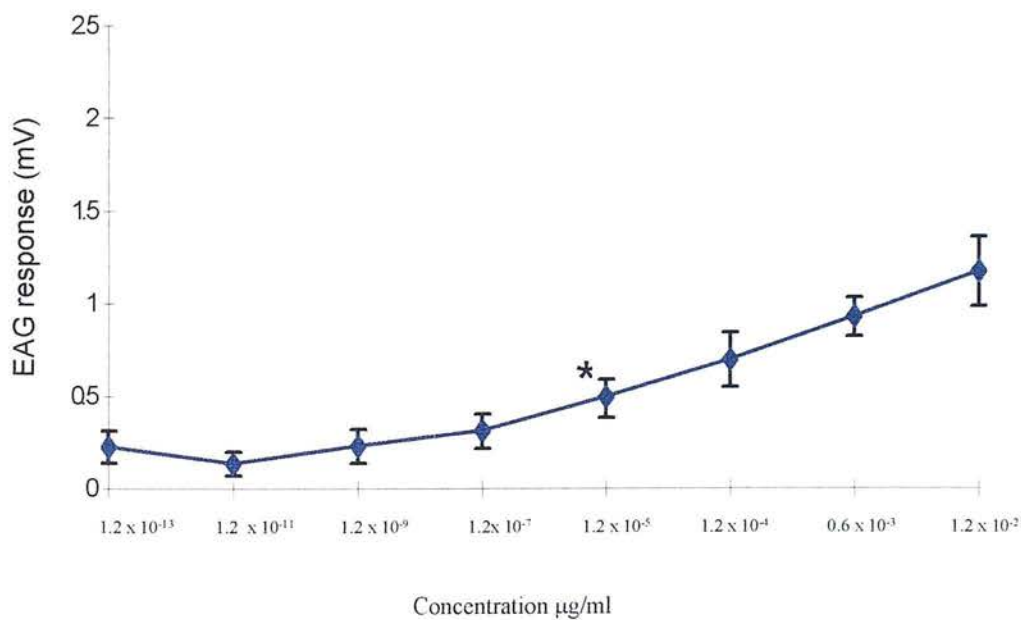


Fig. 4.9 The EAG response in mV (\pm SE) of *P. melanarius* to *cis*-3-hexanol (sample size=12, * = Threshold concentration)

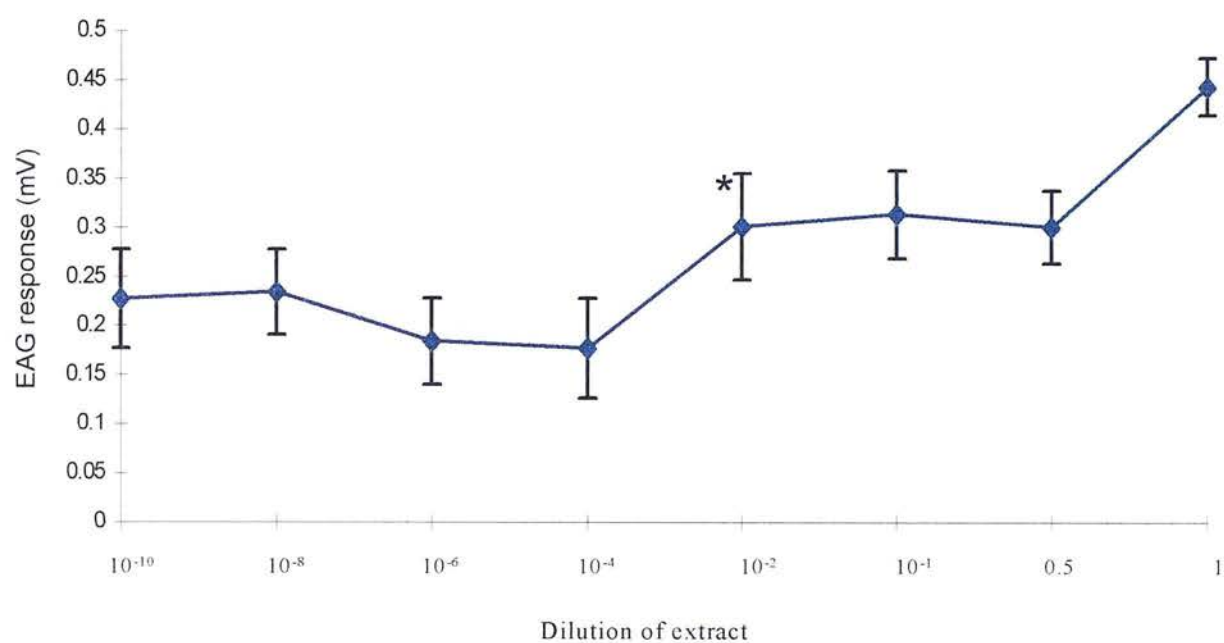


Fig. 4.10 The EAG response in mV (\pm SE) of *P. melanarius* to *Phacelia* flower extract (sample size=12, * = Threshold concentration)

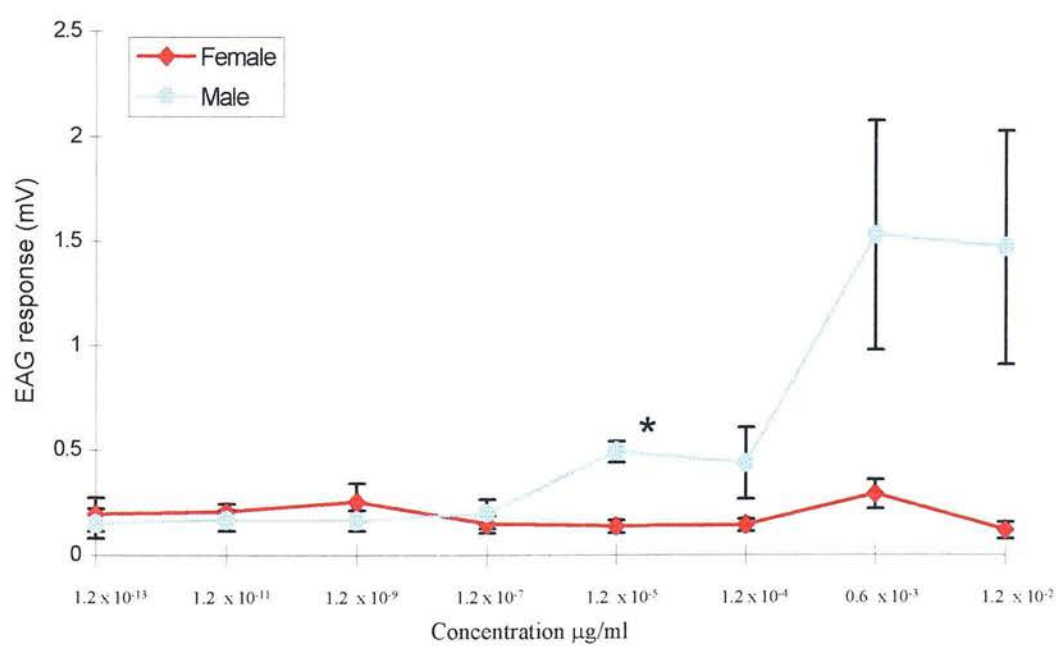


Fig. 4.11 The EAG response in mV (\pm SE) of *P. melanarius* to (E)-β-farnesene (sample size=10, * = Threshold concentration)

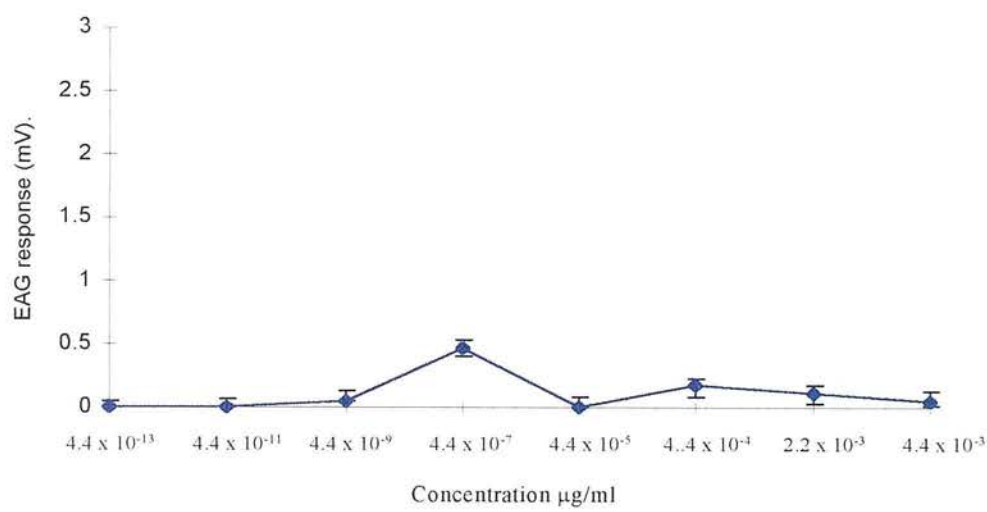


Fig. 4.12 The EAG response in mV (\pm SE) of *N. brevicollis* to acetaldehyde (sample size=12)

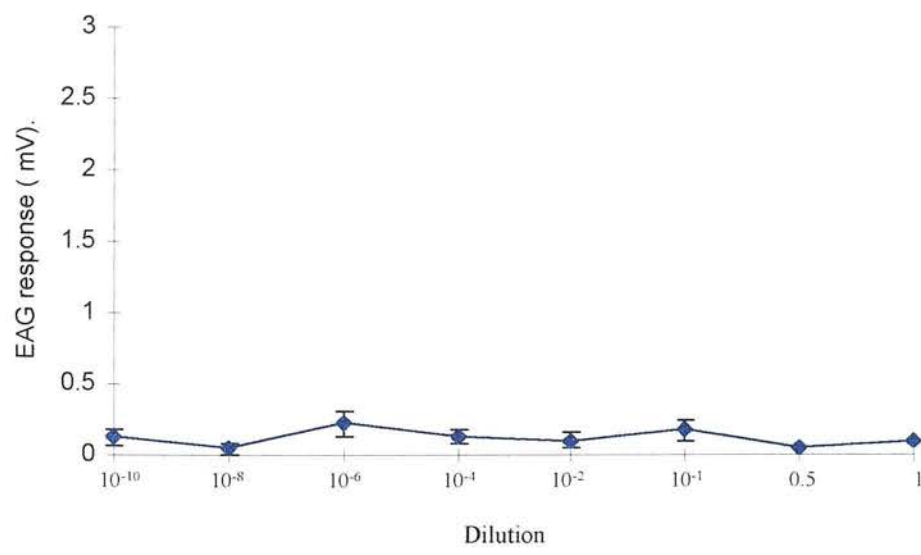


Fig. 4.13 The EAG response in mV (\pm SE) of *N. brevicollis* to artificial aphid honeydew (sample size=12)

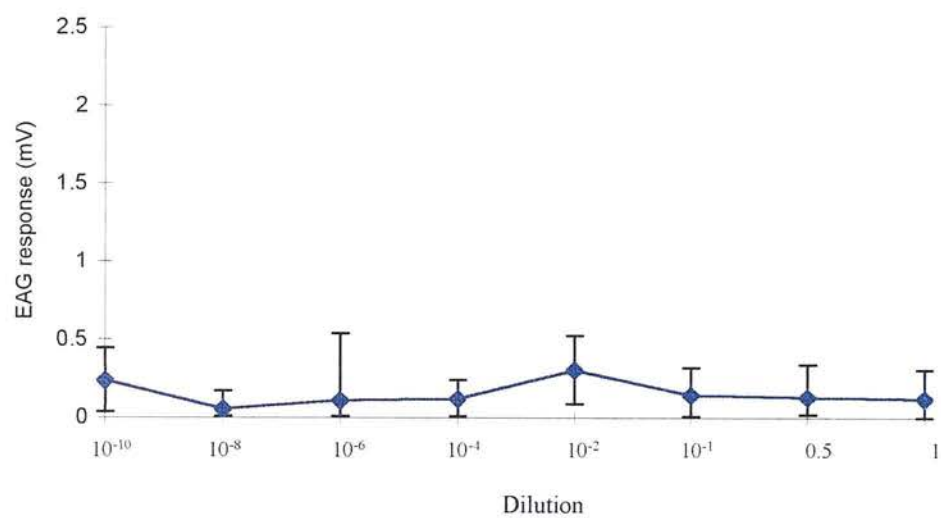


Fig. 4.14 The EAG response in mV (\pm SE) of *N. brevicollis* to β -caryophyllene (sample size=12)

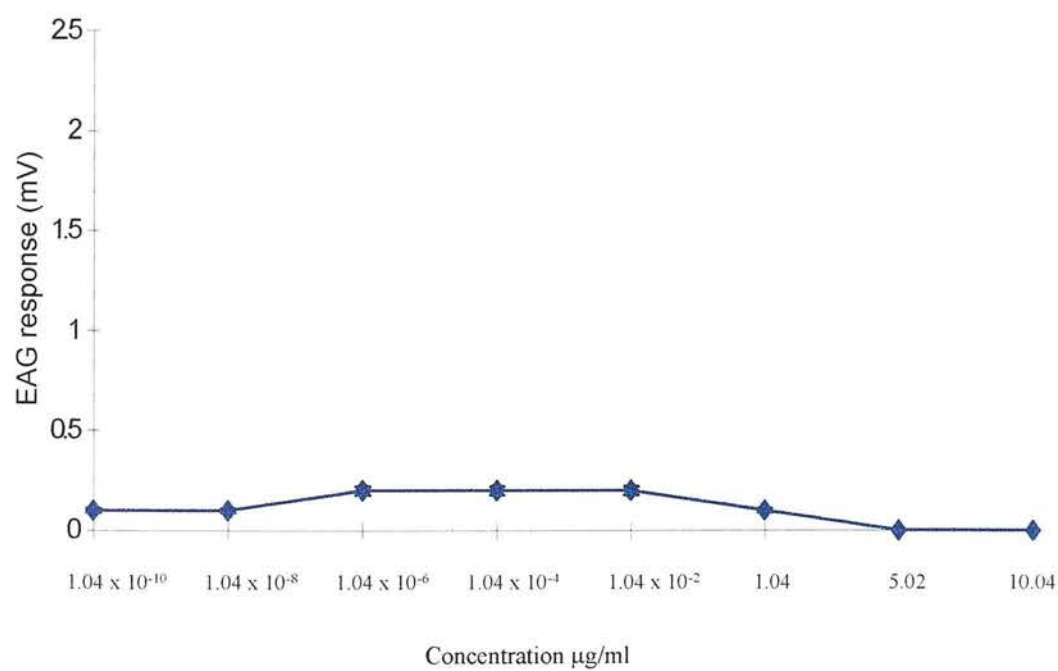


Fig. 4.15 The EAG response in mV (\pm SE) of *N. brevicollis* to β -hydroxybutyric acid (sample size=12)

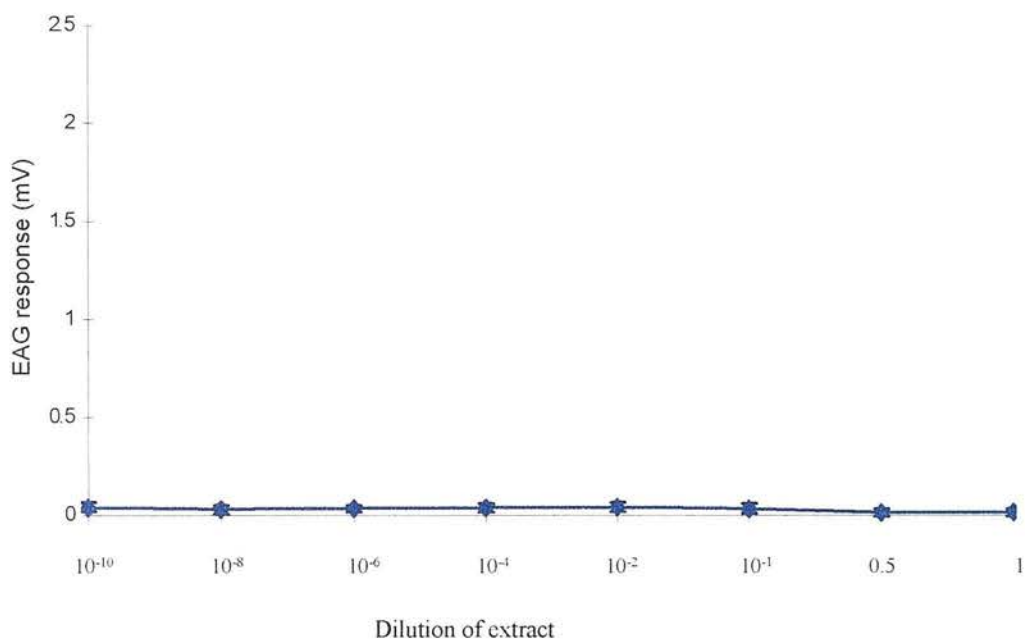


Fig. 4.16 The EAG response in mV (\pm SE) of *N. brevicollis* to *Phacelia* leaf extract (sample size=12)

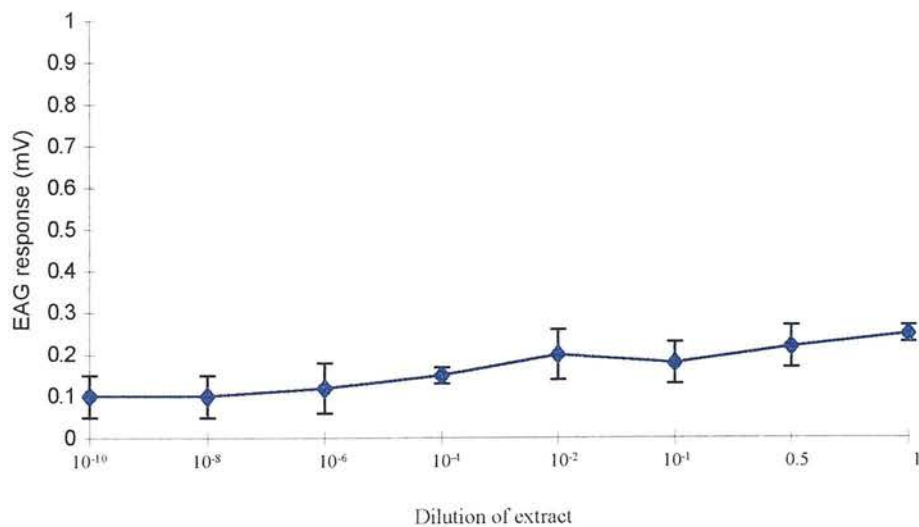


Fig. 4.17 The EAG response in mV (\pm SE) of *N. brevicollis* to *Phacelia* flower extract (sample size=12)

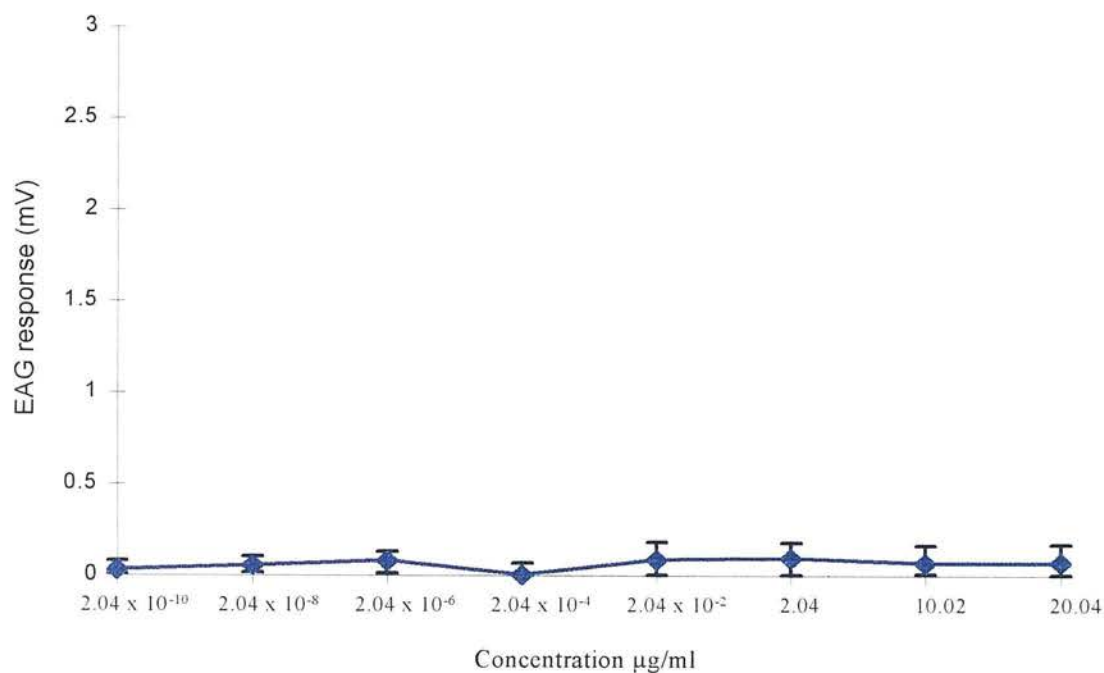


Fig. 4.18 The EAG response in mV (\pm SE) of *N. brevicollis* to L-tryptophan (sample size=12)

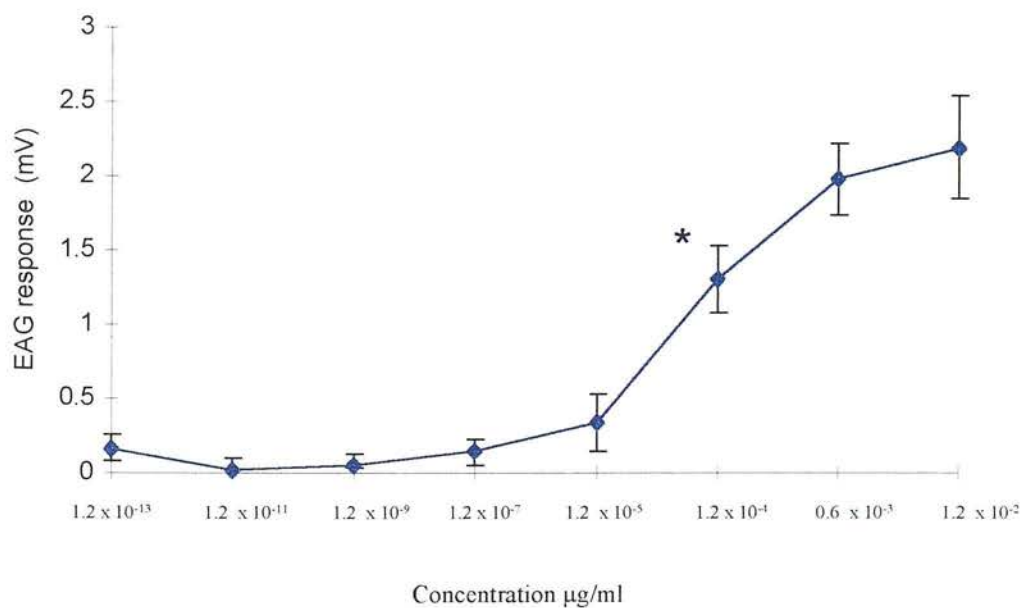


Fig. 4.19 The EAG response in mV (\pm SE) of *N. brevicollis* to cis-3-hexanol (sample size=12, * = Threshold concentration)

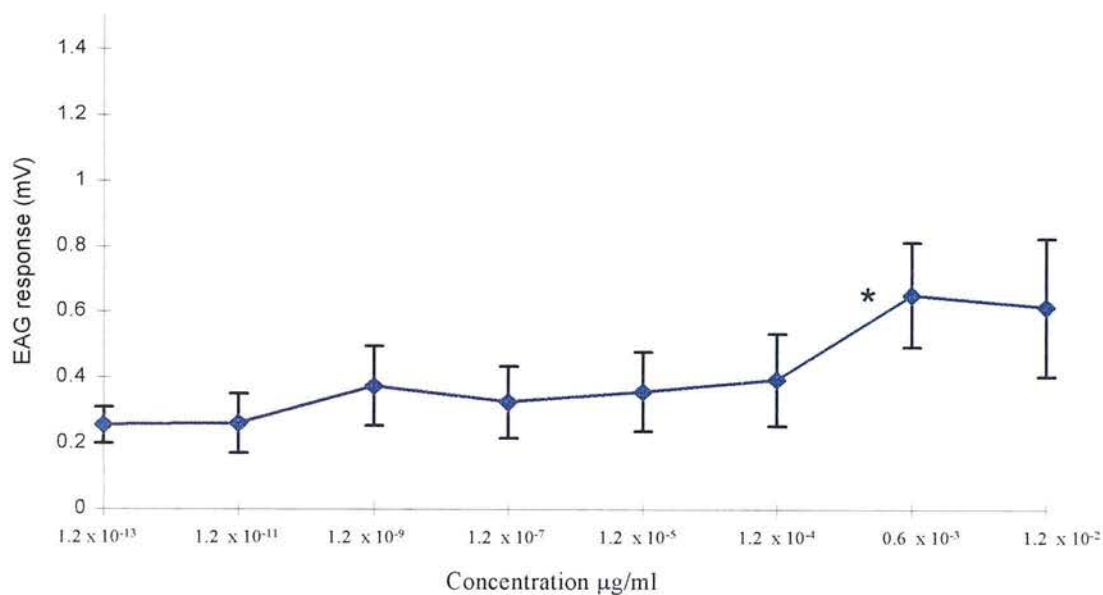


Fig. 4.20 The EAG response in mV (\pm SE) of *N. brevicollis* to (E)-β-farnesene (sample size=12, * = Threshold concentration)

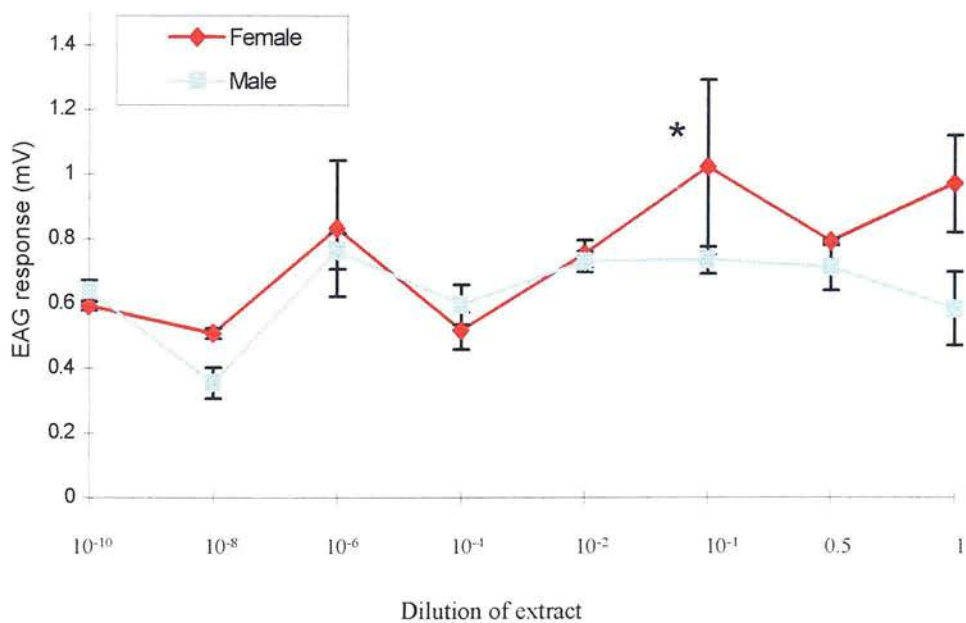


Fig. 4.21 The EAG response in mV (\pm SE) of *N. brevicollis* to *Nepeta* extract (sample size=10, * = Threshold concentration).

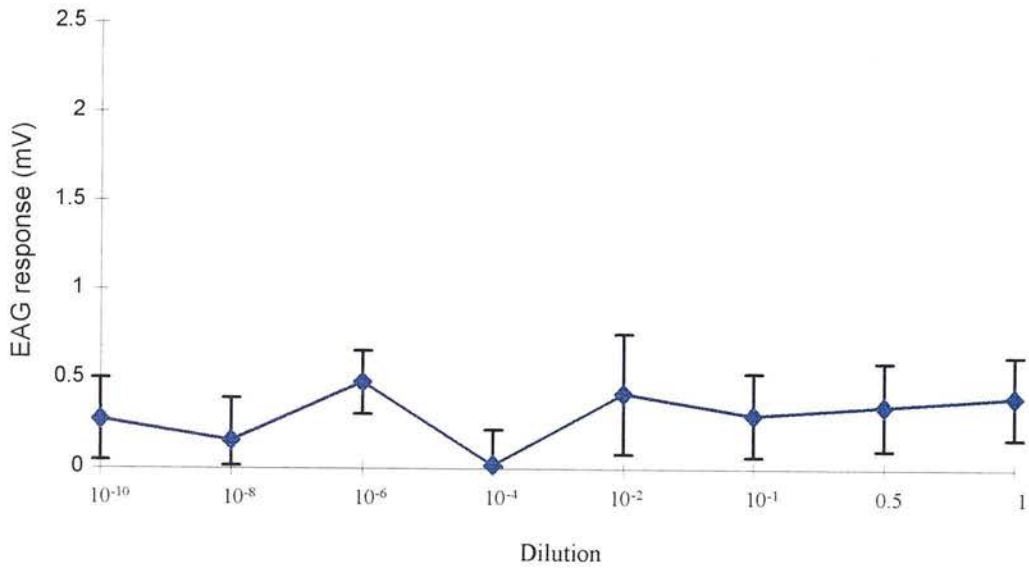


Fig. 4.22 The EAG response in mV (\pm SE) of *E. balteatus* to artificial aphid honeydew (sample size=12)

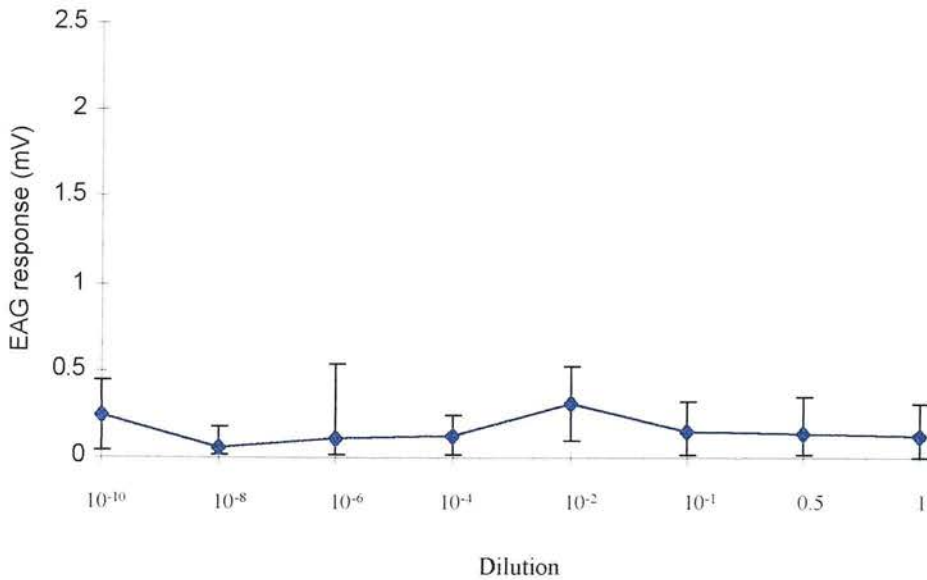


Fig. 4.23 The EAG response in mV (\pm SE) of *E. balteatus* to β -caryophyllene (sample size=12)

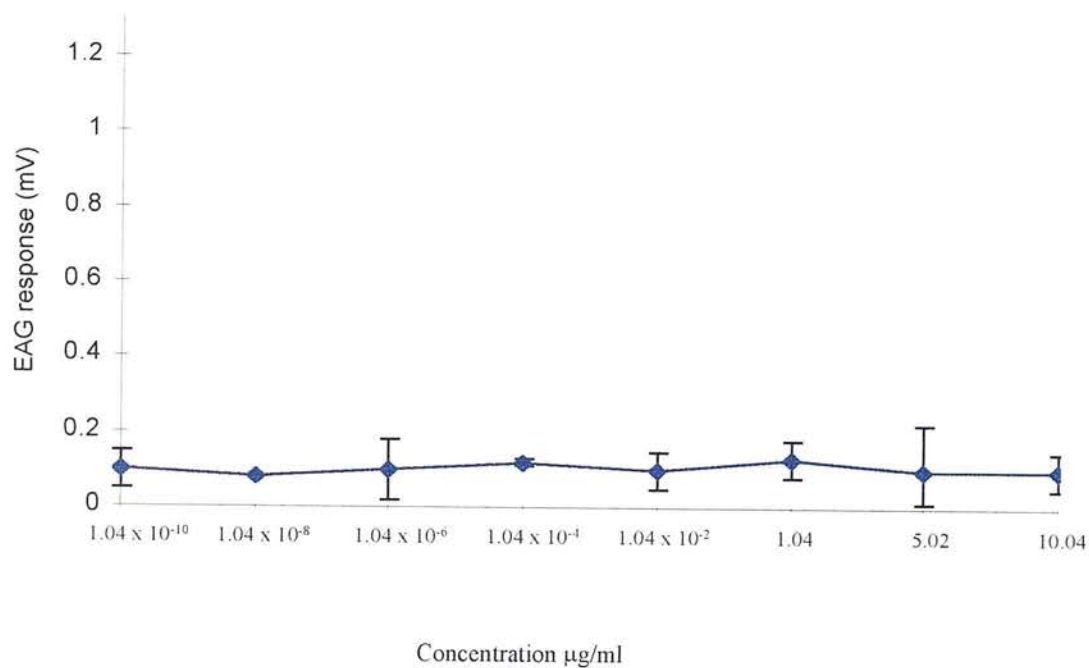


Fig. 4.24 The EAG response in mV (\pm SE) of *E. balteatus* to β -hydroxybutyric acid (sample size=12)

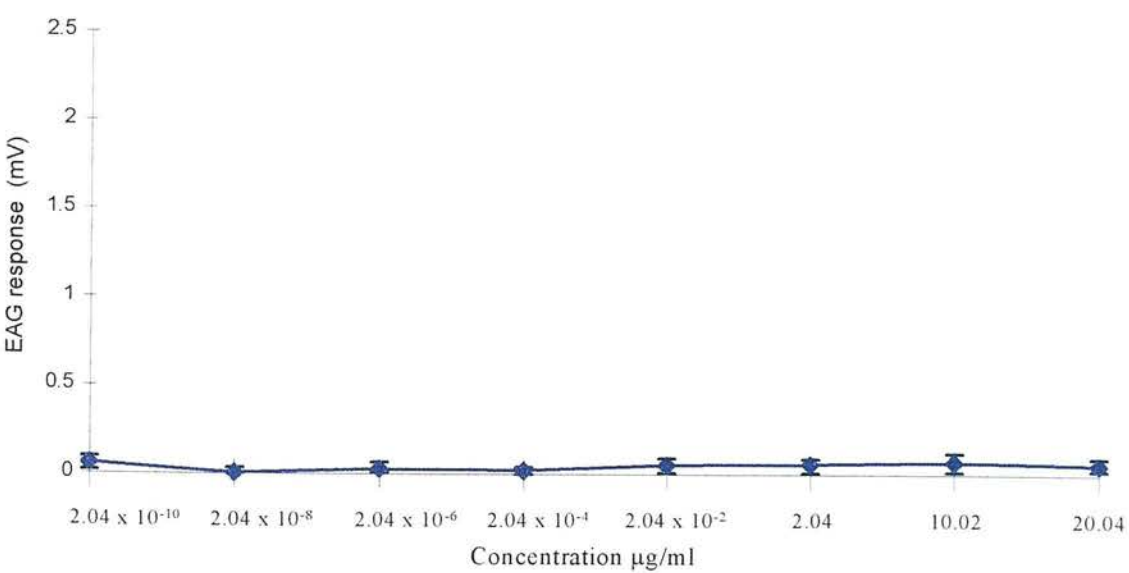


Fig. 4.25 The EAG response in mV (\pm SE) of *E. balteatus* to L-tryptophan (sample size=12)

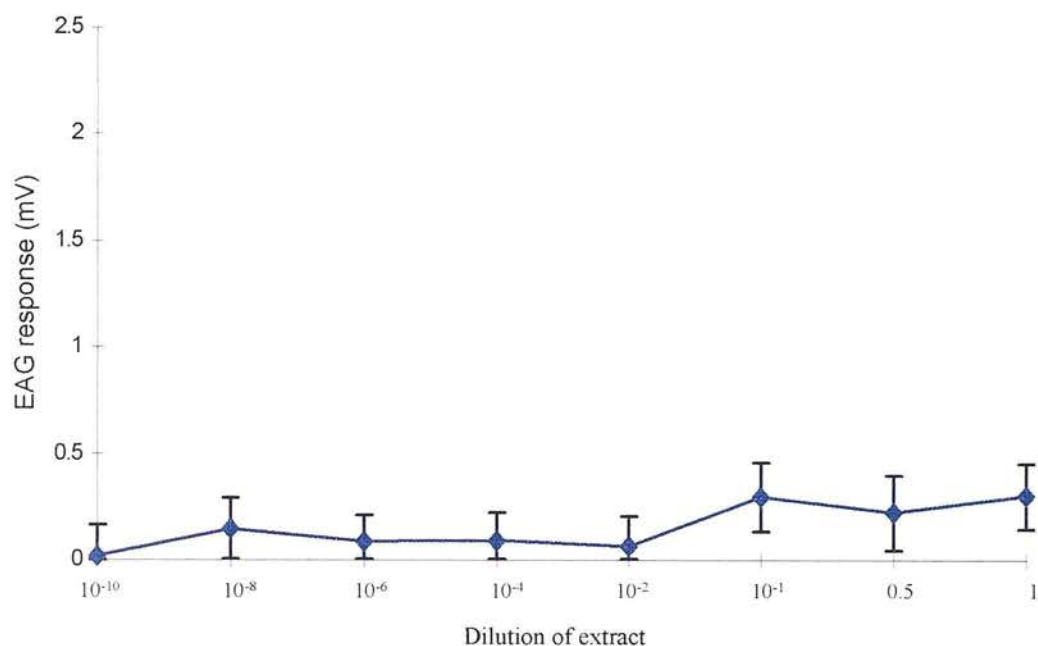


Fig. 4.26 The EAG response in mV (\pm SE) of *E. balteatus* to *Phacelia* leaf extract (sample size=12)

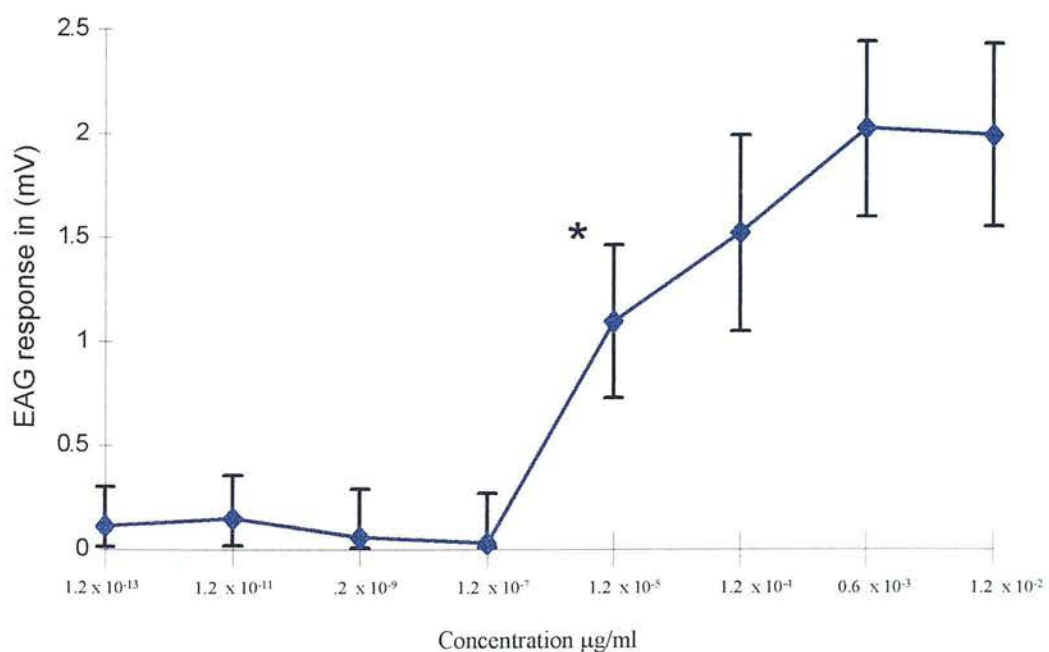


Fig. 4.27 The EAG response in mV (\pm SE) of *E. balteatus* to (E)- β -farnesene (sample size=12, * = Threshold concentration)

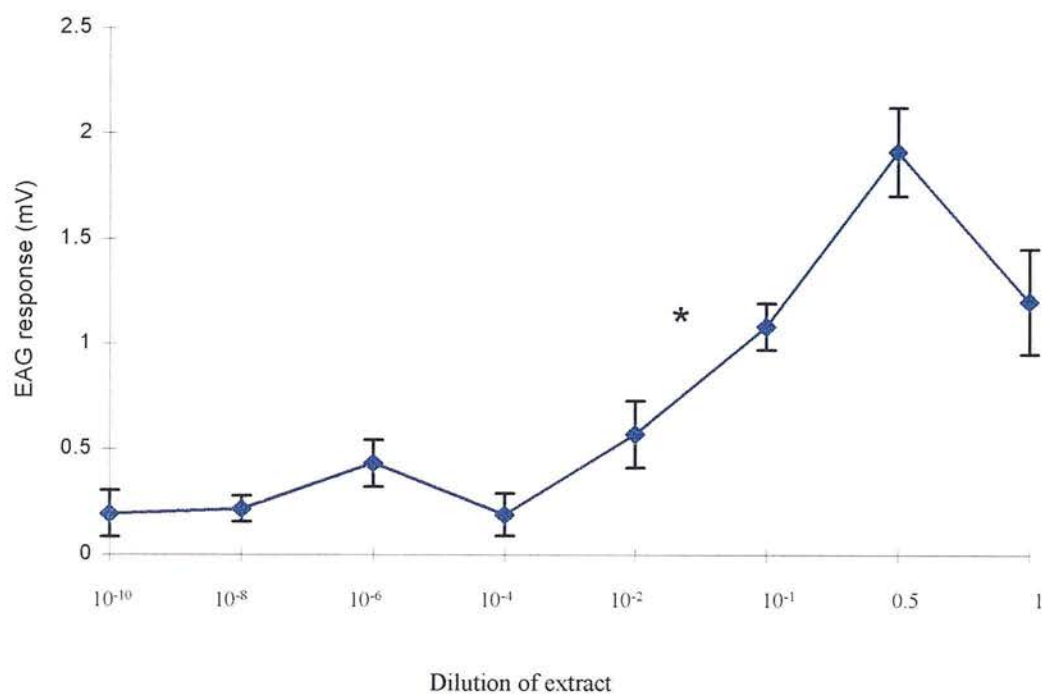


Fig. 4.28 The EAG response in mV (\pm SE) of *E. balteatus* to *Phacelia* flower extract (sample size=12, * = Threshold concentration)

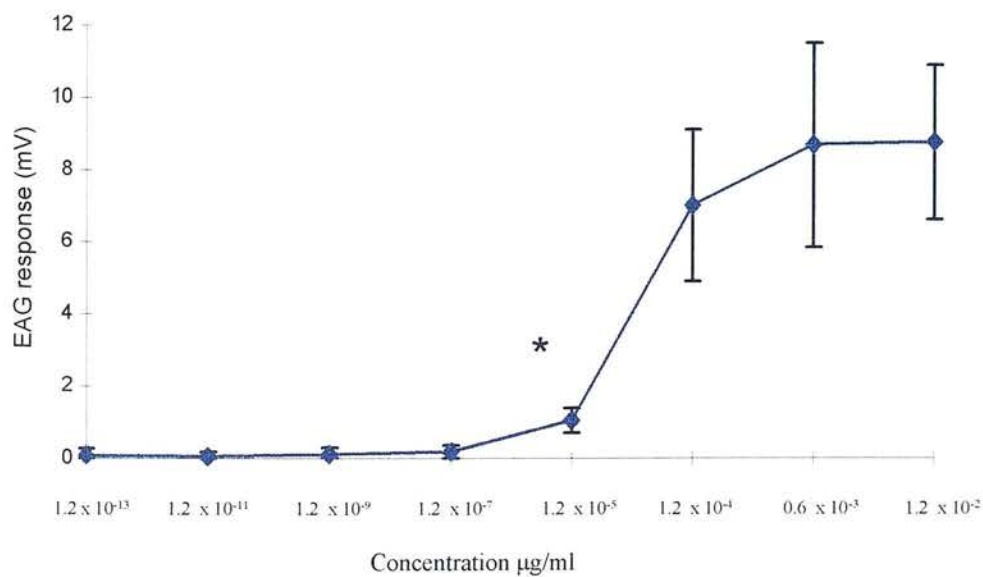


Fig. 4.29 The EAG response in mV (\pm SE) of *E. balteatus* to *cis*-3-hexanol (sample size=12, * = Threshold concentration)

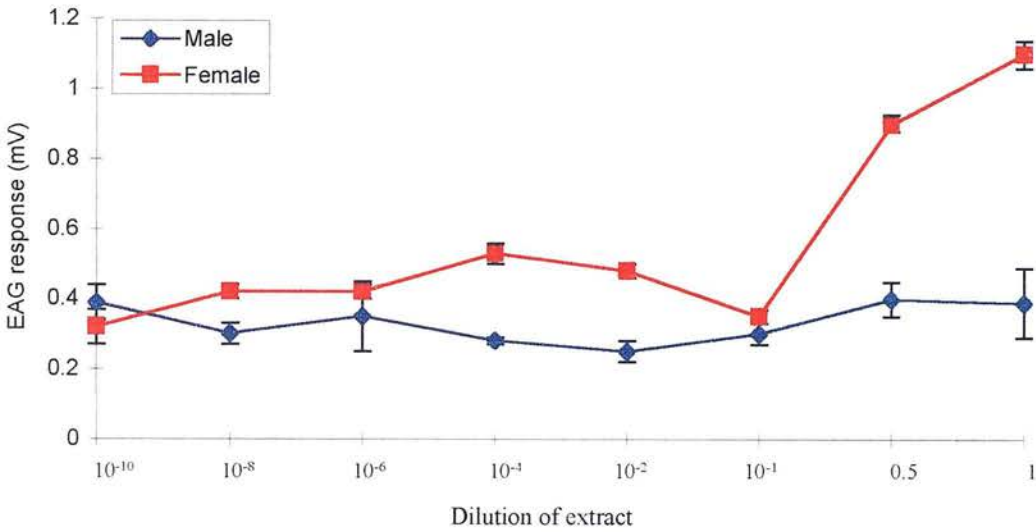


Fig. 4.30 The EAG response in mV (\pm SE) of *E. balteatus* to *Nepeta* extract (sample size=10, * = Threshold concentration)

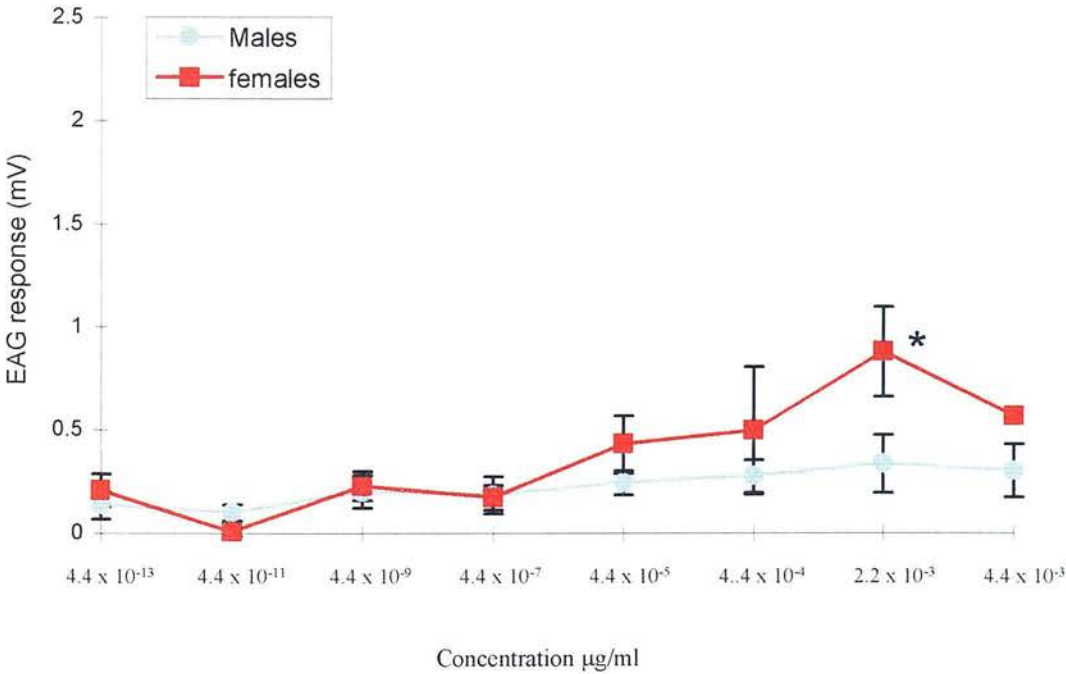


Fig. 4.31 The EAG response in mV (\pm SE) of *E. balteatus* to acetaldehyde (sample size=10, * = Threshold concentration.)

Table 4.4 Threshold concentration for *P. melanarius*

Semiochemical	Threshold dilution	Threshold concentration
cis-3-hexanol (males + females)	10^{-1}	$1.2 \times 10^{-1} \text{ g ml}^{-1}$
(E)- β -farnesene (males)	10^{-2}	$1.43 \times 10^{-2} \text{ g ml}^{-2}$
<i>Phacelia</i> flower extract (males + Females)	10^{-2}	-

Table 4.5 Threshold concentration for *N. brevicollis*.

Semiochemical	Threshold dilution	Threshold concentration
cis-3-hexanol (males + females)	10^{-1}	$1.2 \times 10^{-1} \text{ g ml}^{-1}$
<i>Nepeta</i> extract (females)	10^{-2}	-

Table 4.6 Threshold concentrations for *E. balteatus*

Semiochemical	Threshold dilution	Threshold concentration
acetaldehyde (females)	0.5	$2.2 \times 10^{-3} \text{ g ml}^{-1}$
cis-3-hexanol (males + females)	10^{-4}	$1.2 \times 10^{-5} \text{ g ml}^{-1}$
(E)- β -farnesene (males + females)	10^{-2}	$1.43 \times 10^{-2} \text{ g ml}^{-1}$
<i>Nepeta</i> (females)	10^{-2}	-
<i>Phacelia</i> flowers (males + females)	10^{-2}	-

4.3.2 *N. brevicollis*

Both male and female *N. brevicollis* did not show an electrophysiological response to acetaldehyde, artificial honeydew, β -caryophyllene, β -hydroxybutyric acid, *Phacelia* flower or leaf extract, and L-tryptophan ($P>0.05$, Figs. 4.12-4.18). Both male and female *N. brevicollis* exhibited a dose dependent EAG response to cis-3-hexanol and (E)- β -farnesene ($P<0.05$, and <0.01 respectively Figs. 4.19 and 4.20). However, only female *N. brevicollis* exhibited a significant EAG response to *Nepeta* extract (females $P<0.01$, males $P>0.05$, Fig. 4.21). P values for the EAG responses of *N. brevicollis* are shown in Appendix 1, Table 10.2.

4.3.3 *E. balteatus*

For both male and female *E. balteatus* there was no significant difference between antennal responses to the methanol controls and artificial honeydew, β -caryophyllene, β -hydroxybutyric, L-tryptophan, and *Phacelia* leaf extract ($P>0.05$, figs 4.22-4.26).

For both male and female *E. balteatus* there was a significant difference between the response to the methanol controls and cis-3-hexanol ($P<0.01$, Fig.4.29), (E)- β -farnesene ($P<0.01$, Fig. 4.27) and for *Phacelia* flower extract ($P<0.01$, Fig.4.28). With acetaldehyde and *Nepeta* there was no significant difference between the semiochemical and control responses for males ($P>0.05$, Figs.4.31 and 4.30), but for females the response was significantly greater ($P<0.05$ and $P<0.01$ respectively).

The threshold concentrations, defined as the concentration at which the EAG response to the test semiochemical is significantly higher than the response to methanol, for each insect to the semiochemicals is shown in Tables 4.4-4.6. P values for the EAG responses of *E. balteatus* are shown in Appendix 1, Table 10.3.

4.4 Discussion

The assay confirms that antennae of *E. balteatus*, *P. melanarius* and *N. brevicollis* can detect a range of volatile semiochemicals. The insects are also able to detect changes in concentration of the semiochemicals, with higher receptor potentials occurring with increased concentrations of semiochemical. Detecting different concentrations of the compound may be important to allow the insect to carry out positive chemotaxis, the insect moves up an odour plume following increasing concentrations of a volatile chemical (Warren *et al.*, 1996). Stimulatory semiochemicals include those associated with prey and prey habitat. Therefore the perception of wind born odours may have an important role to play in the foraging behaviour of the predators.

Electroantennograms have been successfully employed in the past to study the antennal response of various species of both Coleoptera (Evans & Allen-Williams, 1992; Finnegan & Chambers, 1993; Shu *et al.*, 1996) and Diptera (Frey *et al.*, 1992; Cosse & Baker, 1996; Voskamp *et al.*, 1998) to a range of semiochemicals. However previous EAG studies of both Coleoptera and Diptera have concentrated on the antennal response of pest species to identify either food plant volatiles or pheromones which could be used in control of the pest (Evans & Allen-Williams, 1992; Cosse *et al.*, 1995; Landon *et al.*, 1997).

Studies carried out on the antennal responses of predatory or parasitic insects, have concentrated on hymenopterous parasitoids (Ramachandron & Norris, 1991; Salom *et al.*, 1992; Scholz *et al.*, 1998), with few EAG studies of predatory arthropods.

The EAG assay demonstrated that *E. balteatus*, *P. melanarius* and *N. brevicollis* could detect green leaf and other plant derived volatiles such as cis-3-hexanol and *Phacelia* flower extract. Detection of plant derived semiochemicals has previously been demonstrated in EAG studies of herbivorous Coleoptera (Dickens *et al.*, 1993), Diptera (Cosse *et al.*, 1995) and in parasitoids (Ramachandron & Norris, 1991). In parasitoids detection of plant derived semiochemicals by the antenna is thought to be associated with host habitat location behaviour (Ramachandron & Norris, 1991), mediating the foraging of parasitic insects for hosts. Antennal detection of a mixture of volatiles from the plant-aphid oviposition complex has been demonstrated in another species of aphidophagous hoverfly, *Metasyrphus venablesi* Cn. (Hood-Henderson & Wellington, 1982), which could detect green leaf volatiles (six carbon alcohols), and volatiles from crushed carnation petals (Hood-Henderson & Wellington, 1982). Therefore detection of plant produced volatiles may help predators locate suitable prey and oviposition habitat.

The antenna of many groups of insects can also detect semiochemicals produced by other insects. This includes the detection of pheromones produced and received by members of the same species, for example to allow insects to aggregate for mating or to exploit a food source (Shu *et al.*, 1998; Scholz *et al.*, 1998). EAG studies have demonstrated that the antennae of some predators respond to the semiochemicals produced by their prey, for example the antenna of *M. venablesi* respond to volatile

semiochemicals released by crushed aphids (Hood-Henderson & Wellington, 1982), *Teretrisoma nigrescens* Lewis, a predatory beetle of the larger grain borer *Prostephanus truncatus* Horn, exhibited a positive antennal response to aggregation pheromone of its prey (Scholz *et al.*, 1998). The pheromones of the prey species act as kairomones mediating foraging behaviour the predator (Nordland and Lewis, 1976).

This supports the findings of the present study, with both *P. melanarius* and *E. balteatus* exhibiting an antennal response to (E)- β -farnesene, the active component of the aphid alarm pheromone (Dawson *et al.*, 1982). Female *N. brevicollis* and *E. balteatus* can also detect volatiles from *Nepeta* extract, of which the major components, nepetalactol and nepetalactone, are the active components of the aphid sex pheromone (Dawson *et al.*, 1990). Differential detection of semiochemicals by the antenna of different sexes of the same predator species may be due to differences in the behaviours of the sexes, which is examined by behavioural assays (Chapters 5 and 6).

None of the predators used in the present study exhibited an antennal response to artificial aphid honeydew, which supports the findings of Hood-Henderson & Wellington (1982), who demonstrated that the antenna of *M. venablei* did not respond to volatiles from aphid honeydews. However the antennae of female *E. balteatus* did respond to acetaldehyde, a component of natural aphid honeydews, but missing from artificial honeydews. This contrasts with the EAG study of Hood-Henderson & Wellington (1982), who found that the antennae of *M. venablei* did not respond to individual components of honeydews. This indicates there are

differences in the cues employed by different species of the same family of predators, which may be due to differences in favoured habitats or oviposition behaviours between species (Frey *et al.*, 1992).

4.5 Summary

- Male and female *P. melanarius* exhibited an antennal response to cis-3-hexanol and *Phacelia* flower extract, males also responded to (E)- β -farnesene.
- Male and female *N. brevicollis* exhibited an antennal response to cis-3-hexanol, females also responded to *Nepeta* extract.
- Male and female *E. balteatus* exhibited an antennal response to cis-3-hexanol, (E)- β -farnesene and *Phacelia* flower extract. Females also responded to acetaldehyde and *Nepeta* extract.

Chapter 5

Olfactometry

Chapter 5. Olfactometry

5.1 Introduction

Behavioural assays using airflow to present the insects with a stream of test chemicals have been employed since 1926 with the development of the Y or t-tube olfactometer (Vet *et al.*, 1983). Since then various olfactometers have been used extensively to study semiochemical induced insect behaviour. The number of insects entering the arm of the T or Y shaped glass tube is counted as a measure of attractiveness of the test odour. However this style of olfactometer has several disadvantages; no distinct odour fields are created in the olfactometer that the insects can enter and leave, so they are unable to make a specific choice (Vet *et al.*, 1983). The walls of the tube may provide a guiding stimulus (Visser & Taanman, 1987), and having entered one of the arms of the olfactometer the insects may be behaviourally trapped by other stimuli and be unable to leave the arm to enter the other arm (Vet *et al.*, 1983).

Because of these disadvantages the basic Y or T tube olfactometer can be modified by the addition of small chambers on the arms which enabled the insects to leave and re-enter the odour fields, however they still only provide two choices for the insect (Jepson & Healy, 1998).

Petterson (1970) introduced the design of the four arm olfactometer, which gave the insects four possible choices of odour within an exposure chamber, by creating four distinct odour fields within the chamber. If the test odour is used in only one of the possible odour fields, this olfactometer has a higher chance of detecting attraction of an insect to the test chemical, since by random chance only 25% of insects should be

found in the odour field compared to 50% in the Y or T tube olfactometer (Vet *et al.*, 1983).

However, the use of these olfactometers also has several disadvantages. The size of the chamber virtually precludes any flight by the insects under study and the sharp boundaries that exist between the odour fields are uncharacteristic of the situation found in the field under natural conditions (Evans, 1991). Because of this, insects may respond by orthokinesis, klinokinesis and chemotaxis as well as by positive movement toward the odour source or positive anemotaxis (Vet *et al.*, 1983; Evans, 1991). However, these olfactometers can be used to measure the relative attraction or repellency of various test odours. (Evans, 1991).

The four armed olfactometer was used to gauge the behavioural response of the carabids *P. melanarius* and *N. brevicollis*. These carabids generally hunt by walking across the ground and vegetation to find their prey, and have seldom been seen to carry out prey location during flight (Luff, 1987). Test odours used in these assays were previously identified as inducing a response in the antennal sensilla of the insects under study during electrophysiological studies (Chapter 5).

5.2 Materials and methods

The olfactometer assays were carried out according to the modified method of Tréfás *et al.* (In press). Insects were obtained by the methods outlined in Chapter 3; 24 hours before each trial beetles were removed from the culture and placed into individual petri dishes with strips of damp filter paper. The beetles were lightly

anaesthetised with CO₂ and sexed according to the method of Haliday (1977). The beetles were left overnight without food to standardise their physiological state.

A four armed air flow olfactometer was used to determine the behavioural responses of carabids to semiochemicals (Vet et 1983; Fig.5). The olfactometer was constructed from clear perspex to allow the behaviour of the beetles to be filmed. The olfactometer had internal dimensions of 20mm in depth, 320mm in narrowest width and 380mm in widest width and 57cm square. The PVC tubing connecting the olfactometer to the odour sources was 6mm internal diameter. The olfactometer was constructed in three main parts to allow it to be cleaned easily, a clear flat plate of perspex provides the bottom, a 2cm thick perspex sheet with a star shape with convex walls cut out into it which provides the main olfactometer chamber, and another flat perspex plate used as the top. The top plate has a 2cm diameter hole drilled in the top to allow the beetles to be placed into the olfactometer. During the assays the hole was covered by a small piece of sliding perspex to provide a seal to ensure the system was airtight and that air was drawn equally down each arm of the olfactometer.

Each layer of the olfactometer was secured by a rubber O-ring in a 2mm trench. This provided a seal to ensure the system was airtight. If there are any leaks the air flow down each arm will not be equal and therefore the four odour fields created in the centre of the olfactometer will be uneven. The three layers of the olfactometer were secured together by the use of 20 steel bolts, five down each side of the olfactometer, these were held securely at the top by steel wing nuts. The olfactometer was placed on a wooden platform painted white to provide contrast and

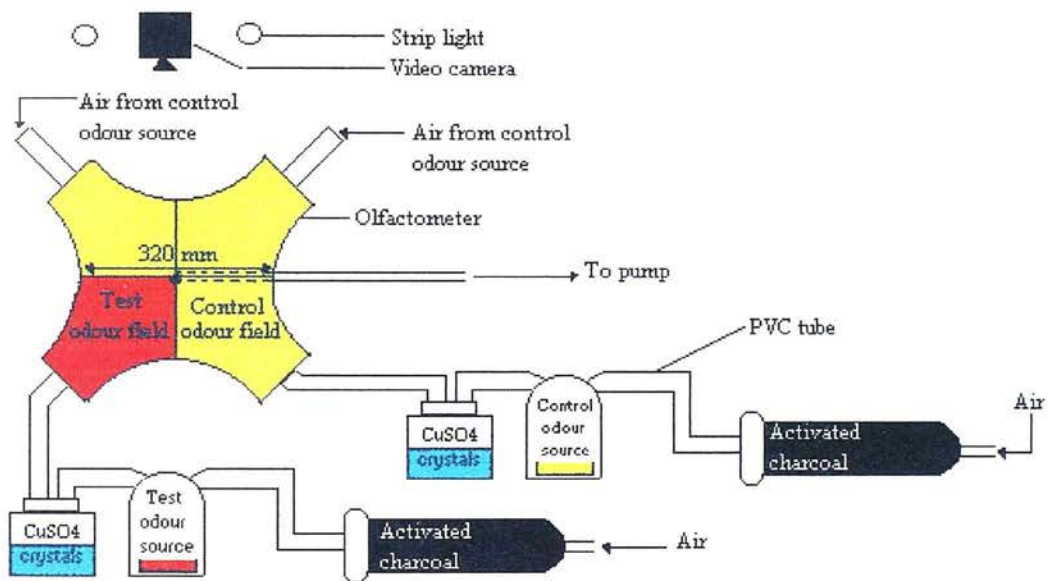


Fig. 5.1 The four armed olfactometer (Tréfás *et al.*, In Press).

enable the dark beetles to be filmed. Lighting was provided by two 6 volt neon strip lights 70cm above the olfactometer. The olfactometer was surrounded by black plastic sheeting and cloth to ensure there was no external visual stimulus.

To create the four odour fields in the olfactometer chamber, air was drawn down each arm and into a central hole in the olfactometer chamber floor. The hole was covered by a 1mm nylon mesh to prevent the beetles from escaping. The hole was connected via PVC tubing to a vacuum pump (Edwards Vacuum Components EB3A, Crawley, UK). The pump provided a continuous and even airflow through the olfactometer chamber. The flow rate of air through each arm of the olfactometer chamber was controlled by means of metal clamps on the PVC tubing connected to the end of each of the arms. Using an air flow meter (Platon Air Products, Basingstoke, UK), air flow through each of the arms was set at 0.35 l s^{-1} , which resulted in four kite shaped odour fields within the chamber. To ensure that all odour fields were even they were visualised using the vapour produced from dry ice to which hot water has been added.

In practice test odours were introduced through one arm of the olfactometer only, resulting in one “test odour” field and three ‘control odour’ fields within the olfactometer chamber. Odour delivery comprised of 50ml of methanol, fully concentrated plant extract or a 10^{-1} dilution of (E)- β -farnesene placed in a Petri-dish in a sealed plastic container connected to the olfactometer arm by PVC tubing. Air was drawn into the olfactometer through activated charcoal to remove any airborne contaminants, and then passed into the container containing the odour source and into the main olfactometer chamber.

Beetles were lightly anaesthetised with CO₂ and placed singly in the centre of the olfactometer chamber. For each trial the beetle was angled to face one of the arms of the olfactometer. With each replicate a fresh beetle was used and was rotated clockwise to face 1 arm on from the previous trial, so that throughout the trial the beetles begin facing all of the arms at least once. Once the beetle regained consciousness, its movements in the olfactometer chamber were filmed for 30 minutes using an overhead monochrome video camera (Sony CCD-IBIS) and recorded on a time lapse video recorder (Panasonic AG640). The time that each beetle spent in each of the odour fields was recorded on an IBM PC using the Observer software program (Noldus Information Technology, Wageningen, The Netherlands).

5.2.1 Semiochemicals

Semiochemicals used in this assay were the methanol control, (E)- β -farnesene, *Phacelia* flower extract and *Nepeta* extract. Before the trials began twenty beetles of each sex were tested in the olfactometer with just methanol in each odour field, to determine that there was no bias to a particular arm in the olfactometer. Twenty beetles of each sex were tested individually for their response to each semiochemical. After five replicates the semiochemical source was moved one arm clockwise so that all four arms were tested to ensure there was no bias. After each replicate, the olfactometer was dismantled and all surfaces were cleaned thoroughly with 80% ethanol to remove any traces of the volatile test odour or semiochemical deposited by the previous beetle.

5.2.2 Statistical analysis of data

Data for each sex of beetle consists of the time that 20 beetles spent in each odour field. The time spent in the semiochemical field and the aggregate time spent in the control fields are not independent as the greater the time spent in the test odour field then the less time will be spent in the control odour field. Nor is the time that the beetles spend in each odour field normally distributed. Consequently analysis of variance which depend on discreet data points is an inappropriate method of analysis. A more appropriate method is to use a randomisation test (Edgington & Gore, 1986; Edgington, 1995 Table, 5.1).

In this experiment data were analysed in a Randomisation test on an IBM PC using the Genstat 5 program (copyright 1995 Lawes Agricultural Trust-Rothamsted Experimental Station). A randomisation test is a permutation test based on randomisation. The test calculates a test statistic (e.g. a T or F statistic) from the experimental data, then randomises the data a set number of times, and for each randomisation a new test statistic is calculated and compared to the test statistic from the experimental data. This tests the null hypothesis that if the test samples are subsamples of the same population and there is no other factor affecting this, i.e. there is no treatment effect, then the individual values will be roughly equal. Therefore it should not matter to which treatment the values are assigned during a randomisation, because the values should be equal so the end calculations should come arrive at a roughly similar answer. The probability of a significant difference occurring between treatments is the number of randomised test statistics equal to or

greater than the experimental test statistic divided by the number of randomisations. If the number of randomised test statistics equal to or greater than the experimental statistic is high the probability that the experimental test statistic occurred by chance is high, and therefore the chance that the experimental test statistic occurred through a treatment effect is low.

The randomisation test used took the mean of the beetle time spent in the semiochemical test odour field and subtracted the mean time spent in each of the control fields over the entire 20 replicates. The contrast, defined as the time each beetle spent in the test odour field minus the time spent in the control odour fields was then calculated for each active beetle in the experimental data and the mean contrast obtained. The data were then randomised 1000 times and the process repeated. The contrast data were then compared to the distribution of contrasts from the randomised data.

5.3 Results

For *P. melanarius* there was no significant bias detected in the control experiments (randomisation test, $P > 0.05$, Figs 5.2 and 5.3). Both male and female *P. melanarius* spent significantly more time in the odour field containing *Phacelia* flower volatiles than in the control odour fields (randomisation tests, males $P < 0.001$, Fig. 5.8, females $P < 0.01$, Fig. 5.9). However, only male *P. melanarius* spent significantly more time in the (E)- β -farnesene odour fields relative to the control odour field

Table 5.1 Randomised linear model for olfactometer data

```
units[40]
open'pmppha.gdt';cha=4
read[cha=4]pha,1,2,3
close cha=4
```

Inputs data into the model in the correct form so subsequent analysis can take place

```
units[160]
variate v
equate !p(pha,1,2,3);v
factor[lev=4;val=40(1,2,3,4);lab=!t(pha,1,2,3)]treat
factor[lev=2;lab=!t(F,M);val=(20(1,2))4]sex
factor[lev=40;val={1...40}4]animal
variate[val=40(-3,1,1,1)]plvpha
scalar ct,tot,totran
```

Divides the data into factors such as treatments, active beetles and sex, and calculates the contrasts

```
for s=1,2
calc ct=0
restrict v,plvpha;cond=sex.eq.s
calc tot=sum(plvpha*v)
```

Calculates the mean contrast and displays it.

```
for i=1...1000
randomize[block=animal/treat]v
calc totran=sum(plvpha*v)
calc ct=ct+(totran<tot)
endfor
```

Randomises the data 1000 times, recalculates the mean contrasts and compares them to the original

```
if s.eq.1
print'Females: randomized control v Phacleia scored higher than observed on'
print [iprint=*] ct,'out of 1000'
endif
if s.eq.2
print [iprint=*] ct,'out of 1000'
endif
endfor
```

Displays the results of for male and female beetles for the treatment.

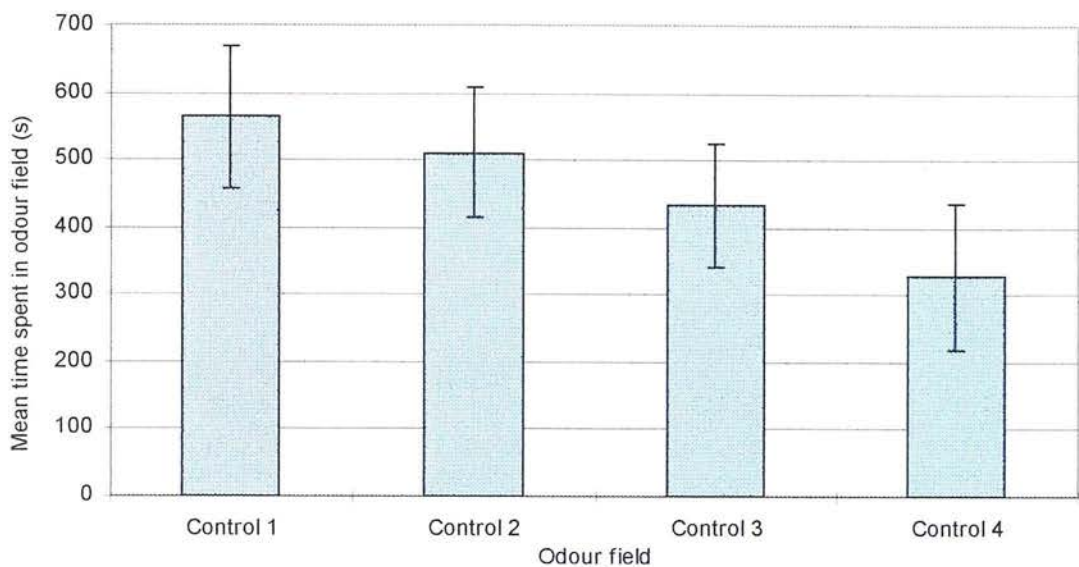


Fig. 5.2 Mean time (\pm SE) male *P. melanarius* spent in each of the odour fields (sample size=20) in the four armed olfactometer with no test semiochemical

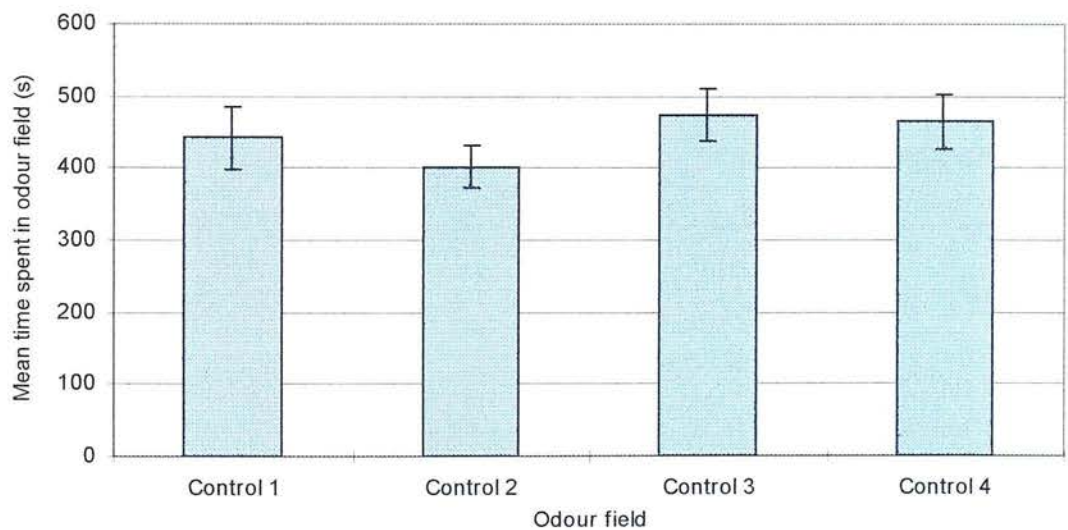


Fig. 5.3 Mean time (\pm SE) female *P. melanarius* spent in each of the odour fields (sample size=20) in the four armed olfactometer with no test semiochemical

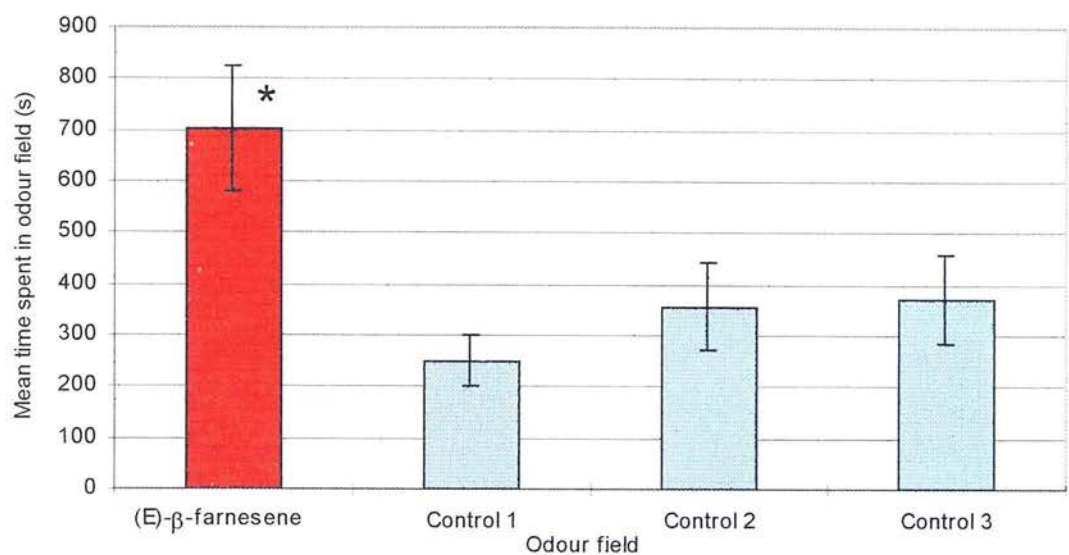


Fig. 5.4 Mean time (± SE) male *P. melanarius* spent in each of the odour fields (sample size=20) in the four armed olfactometer with (E)-β-farnesene as the test semiochemical (* = $P < 0.05$)

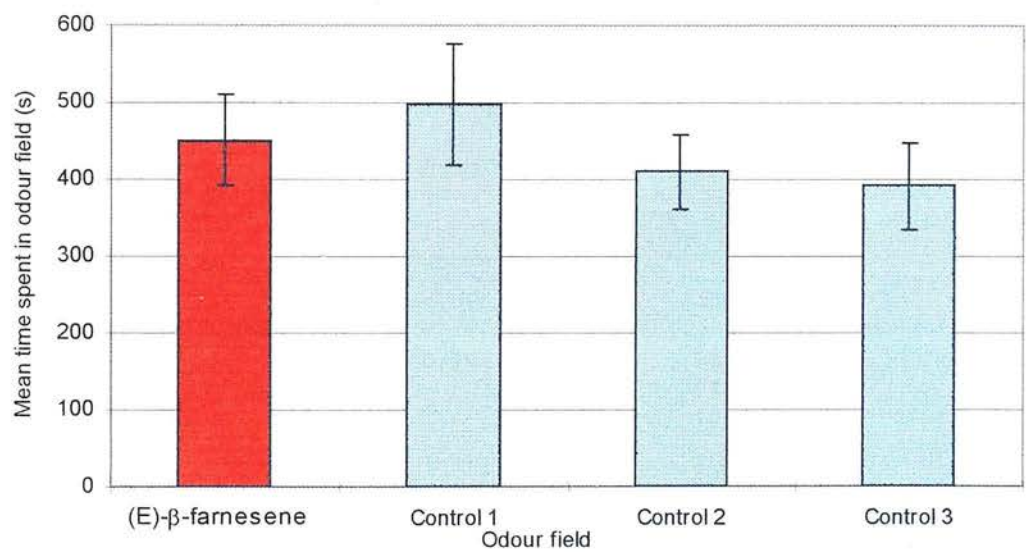


Fig. 5.5 Mean time (± SE) female *P. melanarius* spent in each of the odour fields (sample size=20) in the four armed olfactometer with (E)-β-farnesene as the test semiochemical

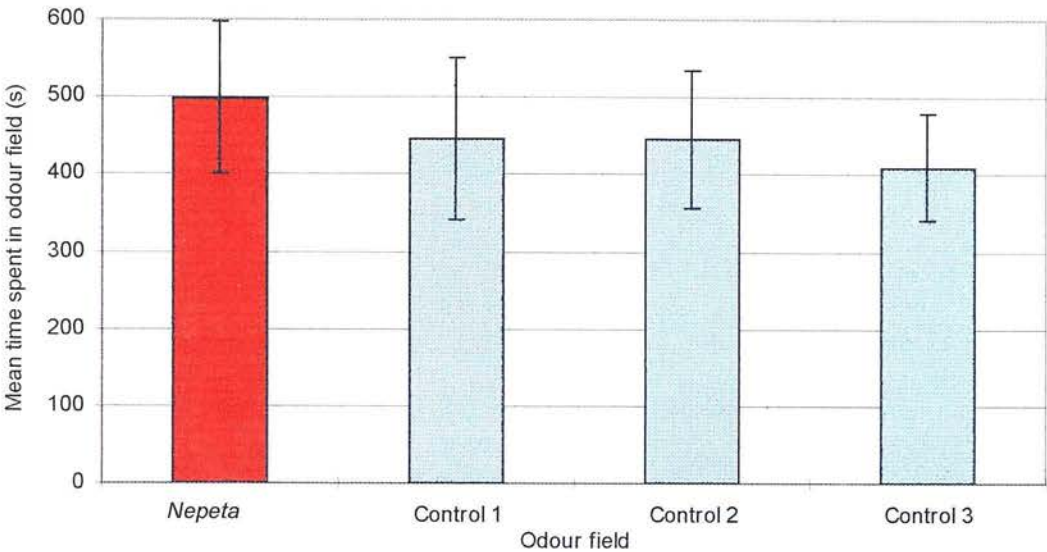


Fig. 5.6 Mean time (\pm SE) male *P. melanarius* spent in each of the odour fields (sample size=20) in the four armed olfactometer with *Nepeta* extract as the test semiochemical

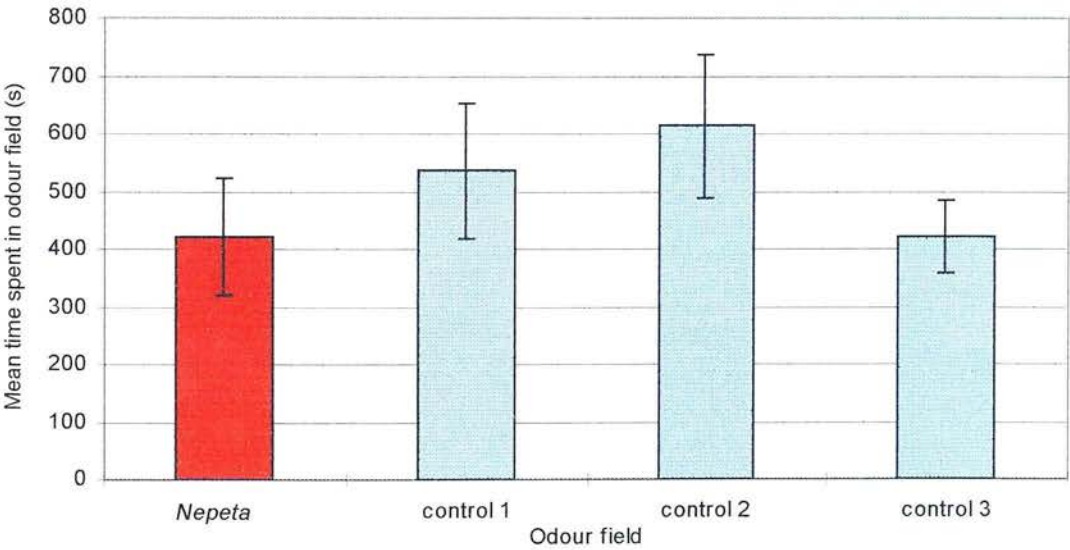


Fig. 5.7 Mean time (\pm SE) female *P. melanarius* spent in each of the odour fields (sample size=20) in the four armed olfactometer with *Nepeta* extract as the test semiochemical

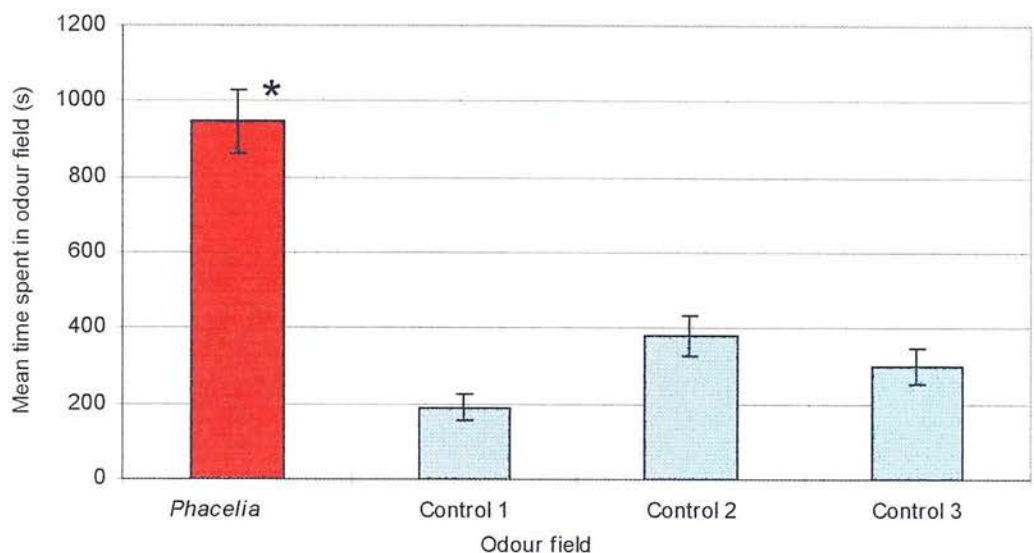


Fig. 5.8 Mean time (\pm SE) male *P. melanarius* spent in each of the odour fields (sample size=20) in the four armed olfactometer with *Phacelia* flower extract as the test semiochemical (* = $P < 0.05$)

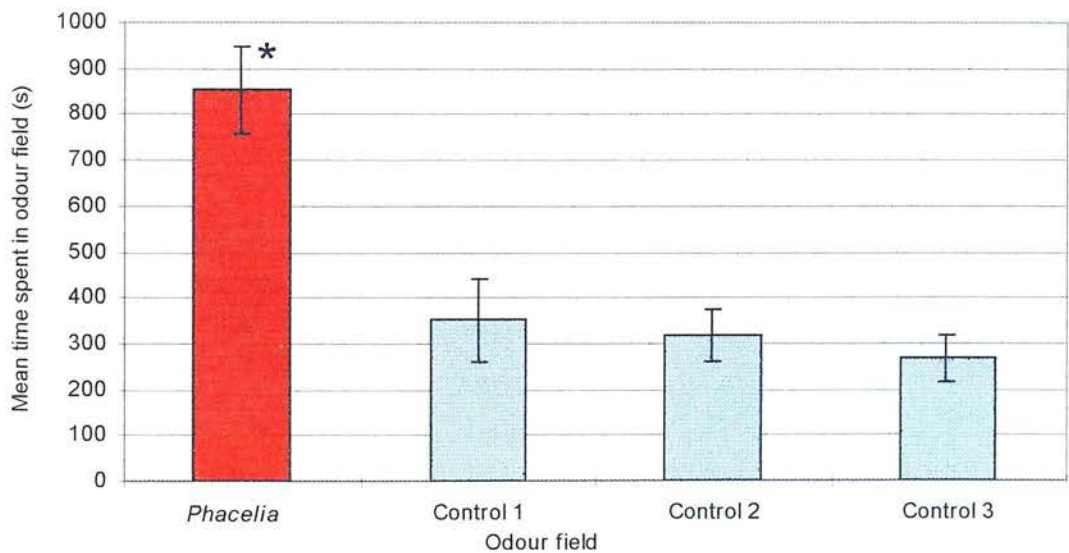


Fig. 5.9 Mean time (\pm SE) female *P. melanarius* spent in each of the odour fields (sample size=20) in the four armed olfactometer with *Phacelia* flower extract as the test semiochemical (* = $P < 0.05$)

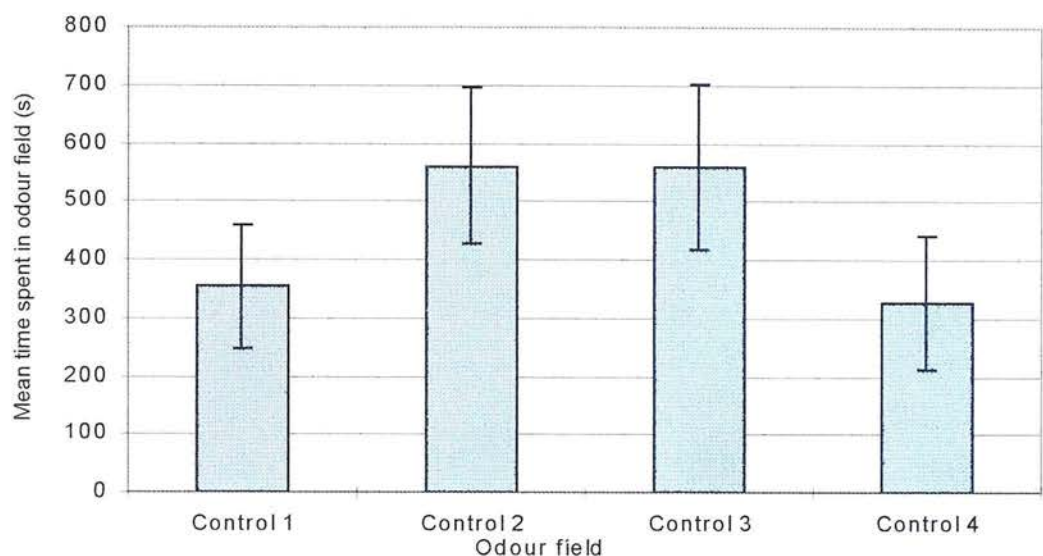


Fig. 5.10 Mean time (\pm SE) male *N. brevicollis* spent in each of the odour fields (sample size=20) in the four armed olfactometer with no test semiochemical

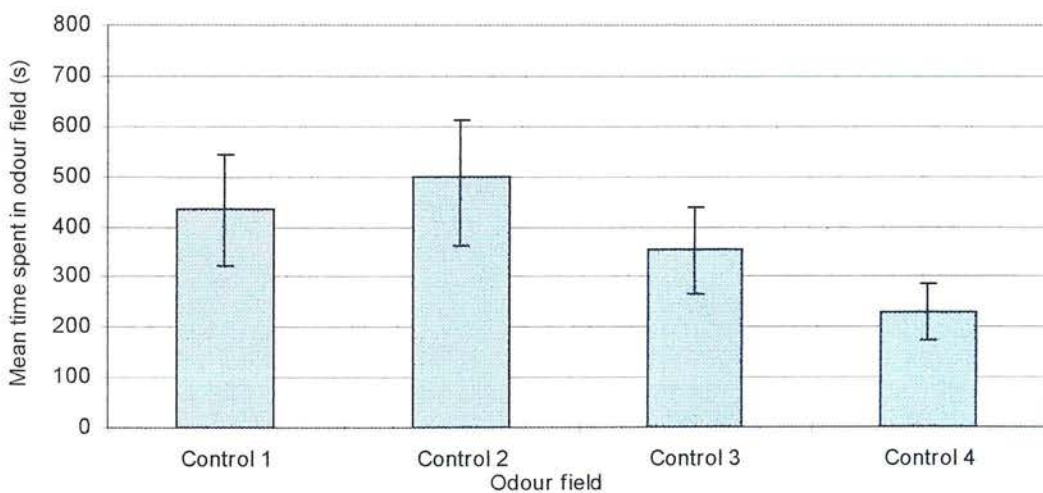


Fig. 5.11 Mean time (\pm SE) female *N. brevicollis* spent in each of the odour fields (sample size=20) in the four armed olfactometer with no test semiochemical.

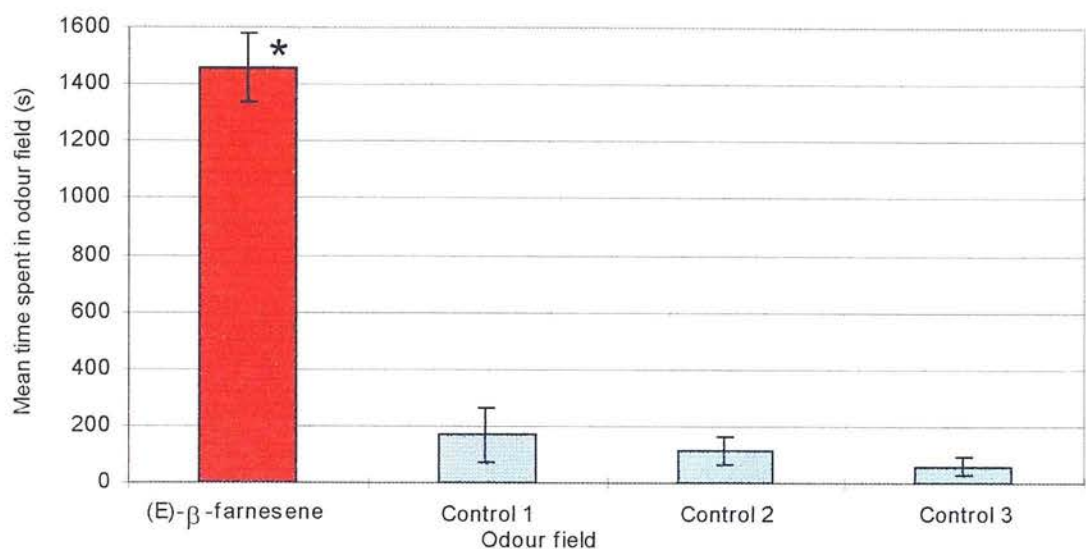


Fig. 5.12 Mean time (\pm SE) male *N. brevicollis* spent in each of the odour fields (sample size=20) in the four armed olfactometer with (E)- β -farnesene as the test semiochemical (* = $P < 0.05$)

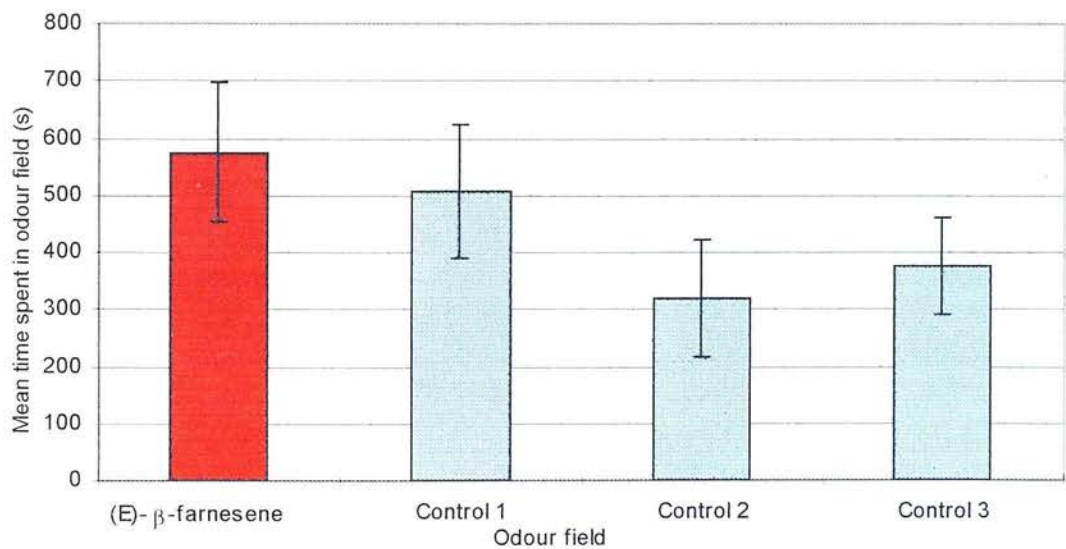


Fig. 5.13 Mean time (\pm SE) female *N. brevicollis* spent in each of the odour fields (sample size=20) in the four armed olfactometer with (E)- β -farnesene as the test semiochemical.

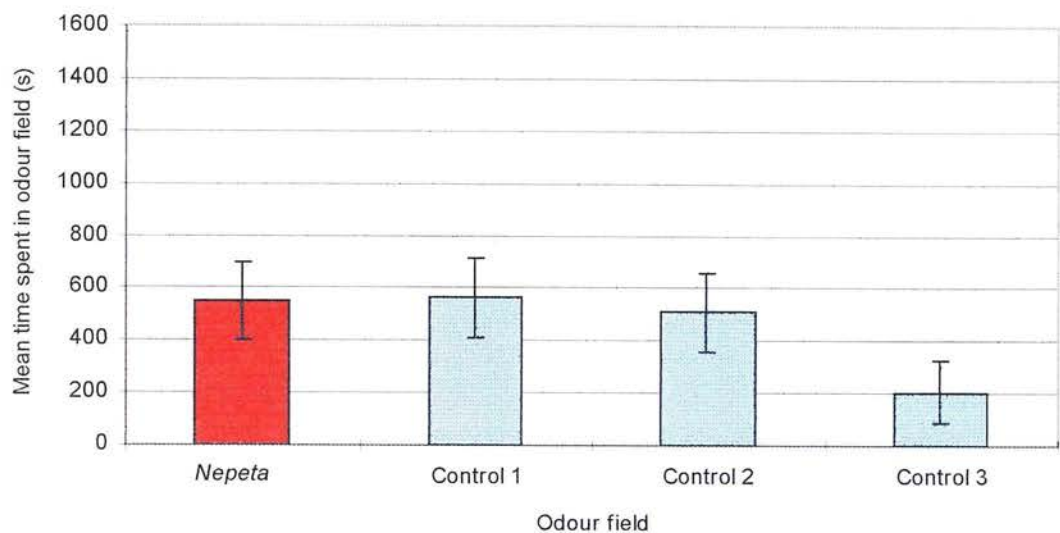


Fig. 5.14 Mean time (\pm SE) male *N. brevicollis* spent in each of the odour fields (sample size=20) in the four armed olfactometer with *Nepeta* extract as the test semiochemical.

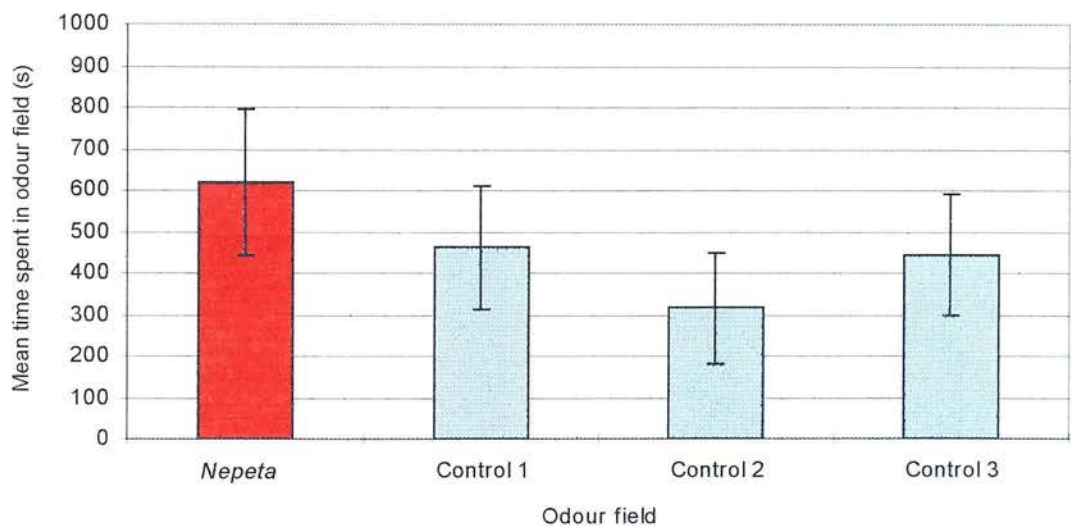


Fig. 5.15 Mean time (\pm SE) female *N. brevicollis* spent in each of the odour fields (sample size=20) in the four armed olfactometer with *Nepeta* extract as the test semiochemical.

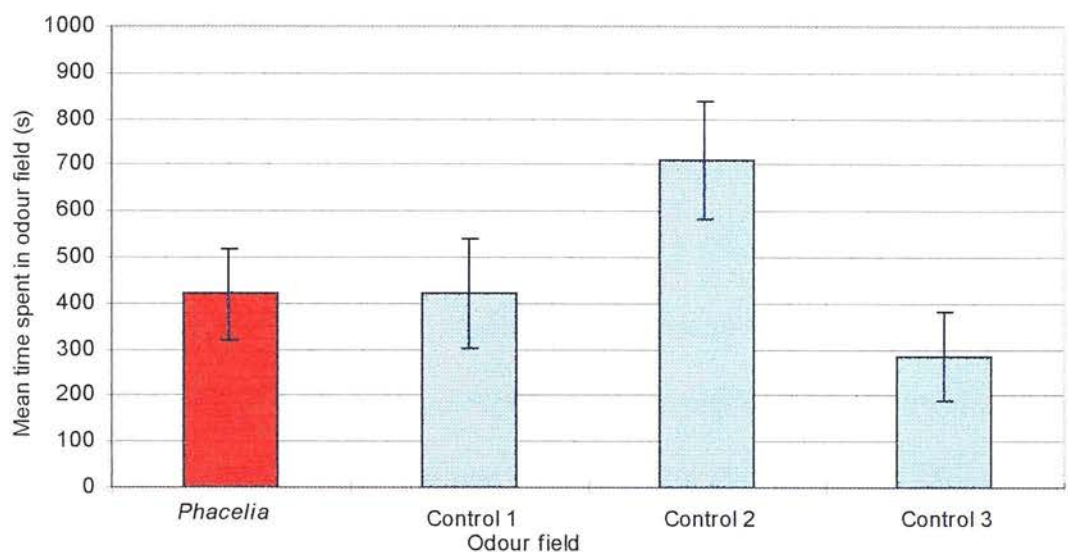


Fig. 5.16 Mean time (\pm SE) male *N. brevicollis* spent in each of the odour fields (sample size=20) in the four armed olfactometer with *Phacelia* flower extract as the test semiochemical

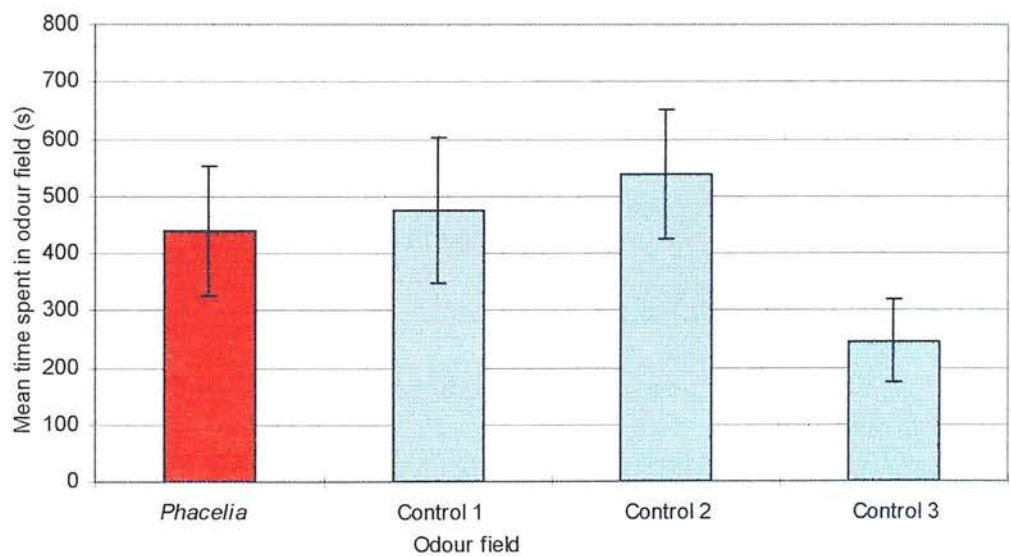


Fig. 5.17 Mean time (\pm SE) female *N. brevicollis* spent in each of the odour fields (sample size=20) in the four armed olfactometer with *Phacelia* flower extract as the test semiochemical.

(randomisation test, males $P < 0.05$, Figs. 5.4 and 5.5). *P. melanarius* showed no significant response to *Nepeta* extract (randomisation test, $P > 0.05$, Figs. 5.6 and 5.7). For *N. brevicollis* there was no significant bias during the control experiments (randomisation test, males $P > 0.05$, Figs 5.10 and 5.11). Male beetles exhibited a significant attractive response to (E)- β -farnesene (randomisation test, $P < 0.01$, Figs. 5.12 and 5.13). *N. brevicollis* did not exhibit a behavioural response to any other test volatile (*Nepeta*, $P > 0.05$, Figs. 5.14 and 5.15; *Phacelia* $P > 0.05$, Figs. 5.16 and 5.17). P values for the olfactory responses of *P. melanarius* and *N. brevicollis* are shown in Appendix 2, Tables 10.4 and 10.5.

5.4 Discussion

The results obtained indicate that adult *P. melanarius* and *N. brevicollis* can detect and move towards sources of volatile semiochemicals. This supports the findings of Wheeler (1989), who suggested that in the nocturnal *Pterostichini* family of carabids, olfactory cues were more important than visual cues. However in *Nebria brevicollis* which is more diurnal in habit (Forsythe, 1987), olfactory cues may be less important than visual cues, as *N. brevicollis* did not show positive orientation to as many cues as *P. melanarius* in the olfactometer.

Attraction to *P. tanacetifolia* in the field was thought to be due to visual cues arising from the *Phacelia* plants i.e. from the colour of the flowers and shapes of the plant rather than from volatile cues (Holland *et al.*, 1994). However, both sexes of adult *P. melanarius* can detect and are attracted to the odour of *Phacelia* flowers in the

olfactometer, therefore, there must be a volatile component to the attraction. *Phacelia* plants provide little in the way of resources for adult carnivorous carabids, although adult *P. melanarius* which have been subject to starvation have been shown to feed on plant material in the form of seeds (Tréfás *et al.*, In Press). *P. tanacetifolia* plants are over 1m tall and large carabid species such as *P. melanarius* do not climb very well (Ekschmit & Wolters, 1997). Therefore *Phacelia* flower resources are out of reach of the beetles. The behavioural assays support the hypothesis that *Phacelia* may provide habitat location cues enabling the beetle to locate suitable habitats where prey might be present before initiating a localised searching behaviour. Prey habitat location behaviour in carabids has been reported in previous studies (Evans, 1988; 1994; Kielty *et al.*, 1995).

Diurnal *N. brevicollis* however probably locate prey habitat through visual cues. This has been demonstrated in other diurnal insects such as *E. balteatus*, with cues such as size density of plant patch and the colour and form of the plants playing a greater role in prey habitat selection than olfactory cues (Chandler, 1966). Olfactory cues only begin to become important in short range prey location (Chandler, 1966). This again gives an indication that *Phacelia* extract is a habitat location rather than a prey location cue. Although female *N. brevicollis* can detect both *Nepeta* extract and (E)- β -farnesene in EAG assays, they do not move toward the chemicals in the olfactometer. This may be because these chemicals only act in synergy with visual cues to increase the efficacy of searching in the beetles, and are ignored if visual cues are not present. Also as the activity of the females falls during the peak of reproductive activity then prey location cues become less important as the female

requires few resources (Niemala & Spence, 1994). The females used in this assay may have been in reproductive condition, and therefore uninterested in prey location cues.

Only male *N. brevicollis* demonstrated any behavioural attraction to volatile semiochemicals in the olfactometer and only to (E)- β -farnesene. (E)- β -farnesene is an important constituent of the alarm pheromone of several aphid species (Dawson *et al.*, 1987). During the reproductive period the male carries out most of the foraging for both food and females and therefore requires resources over this period (Niemela & Spence, 1994). Therefore components of the aphid alarm pheromone may provide a good location cue to find food as it's produced extensively by aphid colonies over this period.

P. melanarius adults have been shown to orient to cues arising directly from prey species such as crickets and blowfly larvae (Wheater, 1989), and from analogues of the pheromone of prey species such as (E)- β -farnesene, a major component of the alarm pheromone of several aphid species (Kielty *et al.*, 1995). This study supports these finding to some extent, however they differ from the study by Kielty *et al* (1995) in that only male *P. melanarius* were attracted to (E)- β -farnesene, with females showing no significant attraction to the compound. This was also the case at the sensory level where females did not exhibit a significant EAG response to (E)- β -farnesene (Chapter 4). During the reproductive period male *P. melanarius* tend to be much more active and move longer distances in search of food or mates as shown by pitfall trap catches (Spence & Niemela, 1994). Females generally locate a patch of

resources early in the season and stay there throughout the reproductive period, thus male *P. melanarius* may possibly use (E)- β -farnesene as a habitat location cue to locate the general area where food and therefore females are located.

5.5 Summary

- Both male and female *P. melanarius* showed positive orientation to *Phacelia* flower extract, while only male *P. melanarius* moved toward sources of (E)- β -farnesene.
- Only male *N. brevicollis* exhibited any behavioural response in the olfactometer to (E)- β -farnesene.

Chapter 6

Wind tunnel assays

Chapter 6. Wind tunnel assays

6.1 Introduction

Wind tunnels have been used extensively for insect behavioural studies (Baker & Linn, 1984). Conditions such as temperature, humidity and wind speed can be modified to provide conditions closer to that experienced in a field situation. This also means that subsequent replicates of the experiment can be carried out under identical conditions (Baker and Linn, 1984).

The behaviour of insects, both flying and walking, has been studied in a variety of different wind tunnels (Visser, 1976; Nottingham and Coaker, 1985). However in each case the design of the wind tunnel remains basically the same, with wind tunnels having three main sections; an effuser section powered by a fan, which draws in the air, brings it to the required conditions and then smoothes the air flow; a test section in which the behavioural responses of the insects can be observed; and a diffuser section where the air is slowed and expelled (Vogel, 1969). The majority also have a regular pattern painted on the floor, over which the insects fly. This provides an optomotor stimulus enabling the insect to gauge and precisely control their movements in the relation to the odour field (David, 1986; Evans, 1991).

Wind tunnels have enabled several types of flight behaviour to be identified in response to insect pheromones (Cardé, 1984) and to plant odours (Miller & Strickler, 1984). The most common response of an insect which detects an odour plume is to fly upwind to try to locate the source of the odour plume; positive odour mediated anemotaxis (Kennedy, 1982; Finch, 1986).

The aims of this study were to identify semiochemicals which induce an attractive behavioural response in *E. balteatus* such as positive anemotaxis. The semiochemicals used in this study were those identified as eliciting a response from the antennal sensilla of *E. balteatus* during electrophysiological assays and include methanol (control), acetaldehyde, (E)- β -farnesene, *Nepeta* leaf extract and *Phacelia* flower extract.

6.2 Materials and methods

The behavioural responses of *E. balteatus* were investigated using a wind tunnel (Hern 1997). The wind tunnel consisted of three main compartments, and effuser divided into two chambers, a transition section with splitters and guide vanes to smooth out the air flow and a settling chamber, with a wide angled diffuser to provide a laminar air flow over the entire testing chamber, the testing chamber itself, and a diffuser section to expel the air. The main testing chamber was 2.0m wide, 1.75m long and 1m high. Alternate black and white stripes 2cm wide were painted across the floor to provide a uniform ground pattern as an optomotor stimulus for the orientation of flying hoverflies (Fig. 6.1).

Lighting was provided by eight evenly spaced fluorescent strip lights suspended 10cm above the clear perspex top of the wind tunnel chamber. This however did provide slight variations in light intensity over the surface of the wind tunnel of approximately 50 lux. The end walls of the wind tunnel consisted of steel chicken wire supporting a 1mm nylon mesh that prevented the insects from escaping but.

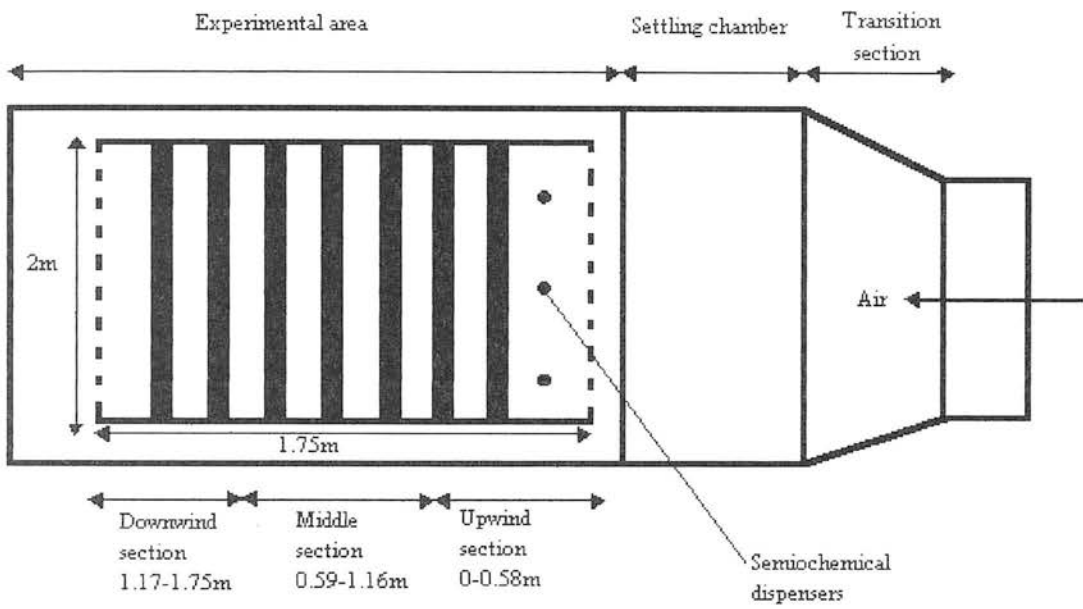


Fig.6.1 Diagrammatic representation of the wind tunnel used in this study
(modified from Hern, 1997).

allowed air to flow freely throughout the chamber. The side walls were clear perspex to allow the behaviour of the insects in the chamber to be monitored. The side walls were hinged at the top to allow easy access to introduce the insects and for ease of cleaning. The other sections consisted of a transition section containing a wide angled diffuser with adjustable splitters and guide vanes to provide an even air flow over the entire test chamber. The third section was a settling chamber with a combination of screens and honeycombs to provide a laminar airflow to the entrance of the experimental chamber. The Wind tunnel was fed by a non-overloading centrifugal blower fan which was used to provide a quality air flow at a constant rate. Ambient air was drawn into the fan from outdoors and heated by 2 electric heaters with a maximum heating output of 96 kilowatts allowing a constant temperature of up to 30°C to be maintained within the test chamber at windspeeds of up to 3 m/sec. Throughout the behavioural assays, wind speed was maintained at 1.0 ± 0.1 m/sec and temperature at $20 \pm 2^\circ\text{C}$.

Hoverflies were cultured in the laboratory, and 24 hours before the experiment, hoverflies were removed from the culture and sexed (Chapter 3). The hoverflies were placed in single sex containers containing damp filter paper but without access to food. This was to standardise the physiological state of the insects, and ensure that they were starved for at least 24 hours. Ten adult flies were introduced into the test chamber and allowed to acclimatise for 30 minutes before the experiment began. Approximately 25 minutes into the acclimatisation period three 100ml plastic wicked dispensers, with their lids pierced to allow 10cm of the 20 cm wick into the container, were placed 0.5m apart across the upwind edge of the test chamber. Each

dispenser contained 50 ml of either methanol control, fully concentrated plant extracts or 1.0 M acetaldehyde solution in distilled water.

After the full 30 minutes of acclimatisation, the behaviour and position of each of the insects in the chamber was recorded every 10 minutes for 90 minutes. The chamber was arbitrarily divided into three sections, the upwind area, 0.0-0.58m away from the semiochemical dispensers, the middle area, 0.58-1.16m from the dispensers and the downwind area, 1.16-1.75m from the dispensers. Behaviour was also divided into three main activities, resting, flight, and landing on the dispensers themselves (Hern, 1997).

The assay was repeated three times for each sex of insect and for each semiochemical used, with 10 fresh insects being used for each replicate. The data were converted into a percentage of insects carrying out a specific behaviour, and transformed using a squareroot arcsine transformation to meet the assumptions of the analysis of variance (Sokal & Rohlf, 1981) and analysed using a two way analysis of variance taking the response of the insects to the methanol alone as the control.

6.3 Results

For both male and female *E. balteatus* there was a significant difference between the behaviours exhibited with the methanol control and acetaldehyde (Fig. 6.2 and 6.3, male and female, $P < 0.01$), (E)- β -farnesene (Fig. 6.4 and 6.5, males and females, $P < 0.01$), and *Phacelia* extract (Fig. 6.6 and 6.7, males and females, $P < 0.01$). Except for the response of male *E. balteatus* to acetaldehyde and (E)- β -farnesene, there were

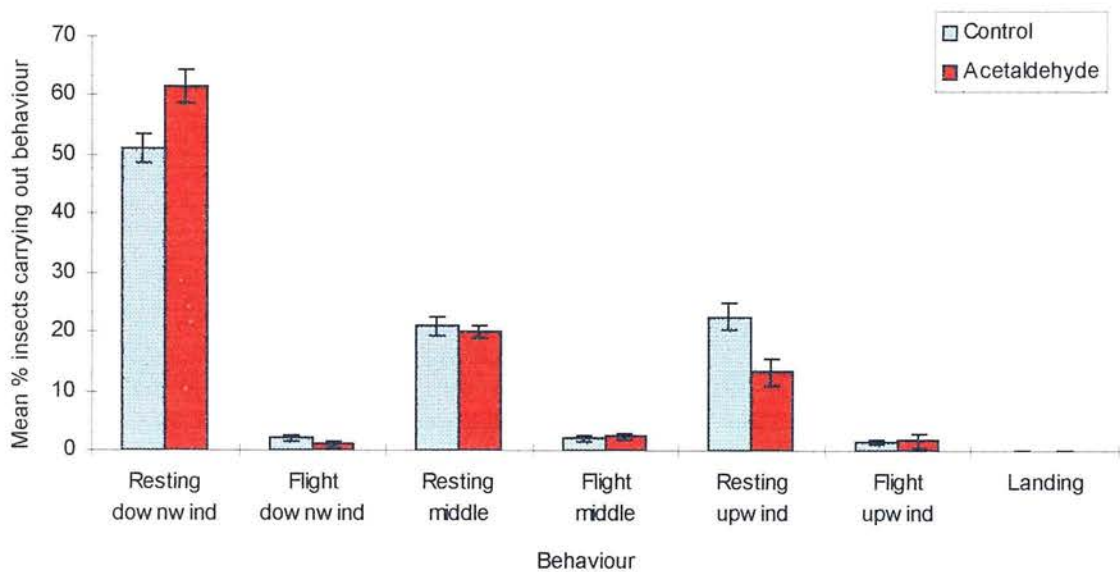


Fig 6.2 Mean % of male *E. balteatus* (\pm SE) carrying out specified behaviour in the wind tunnel with acetaldehyde as an odour source (sample size=3)

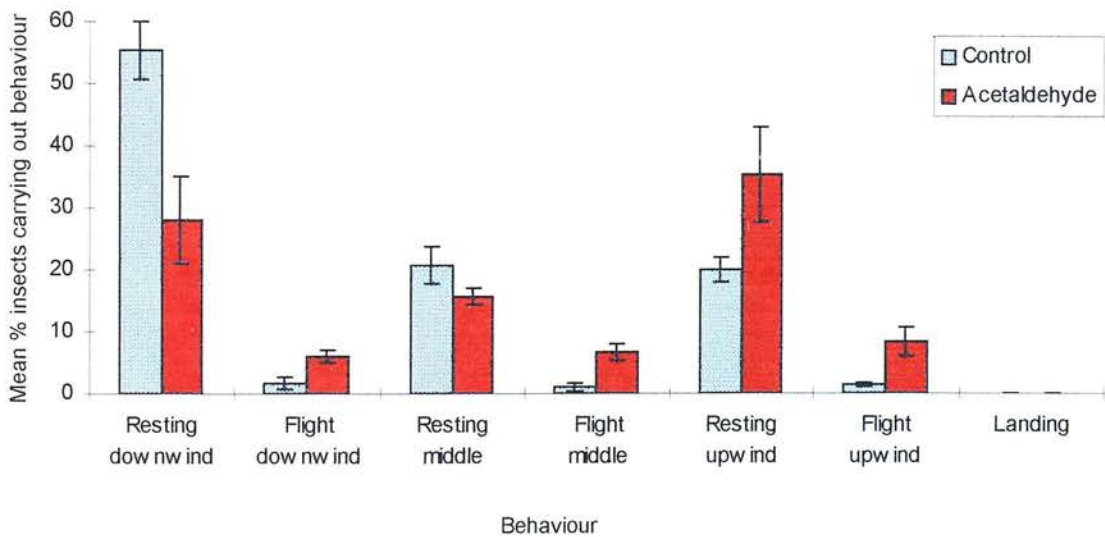


Fig 6.3 Mean % of female *E. balteatus* (\pm SE) carrying out specified behaviour in the wind tunnel with acetaldehyde as an odour source (sample size=3)

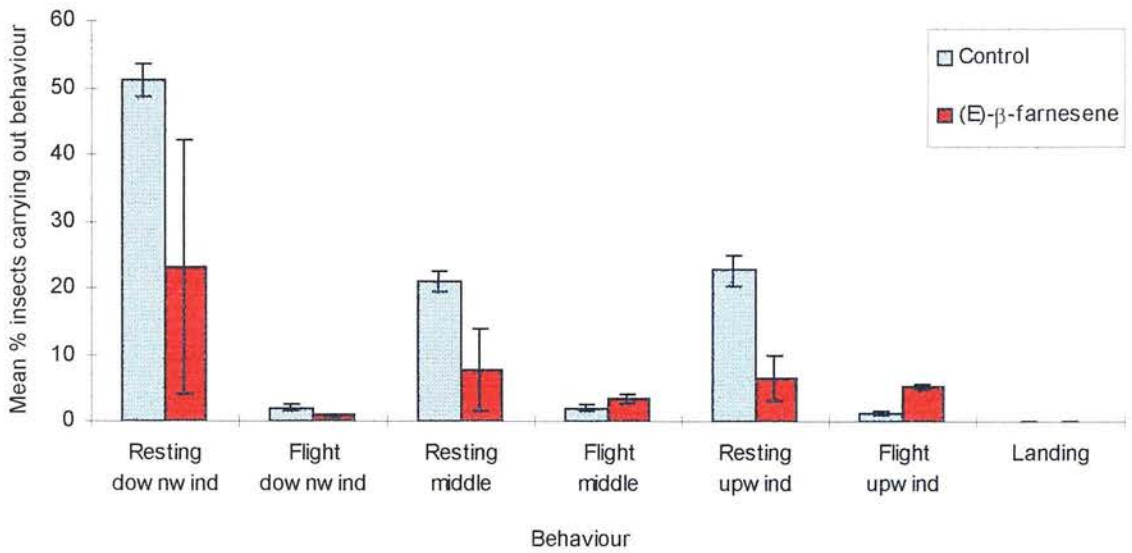


Fig 6.4 Mean % of male *E. balteatus* (\pm SE) carrying out specified behaviour in the wind tunnel with (E)-β-farnesene as an odour source (sample size=3)

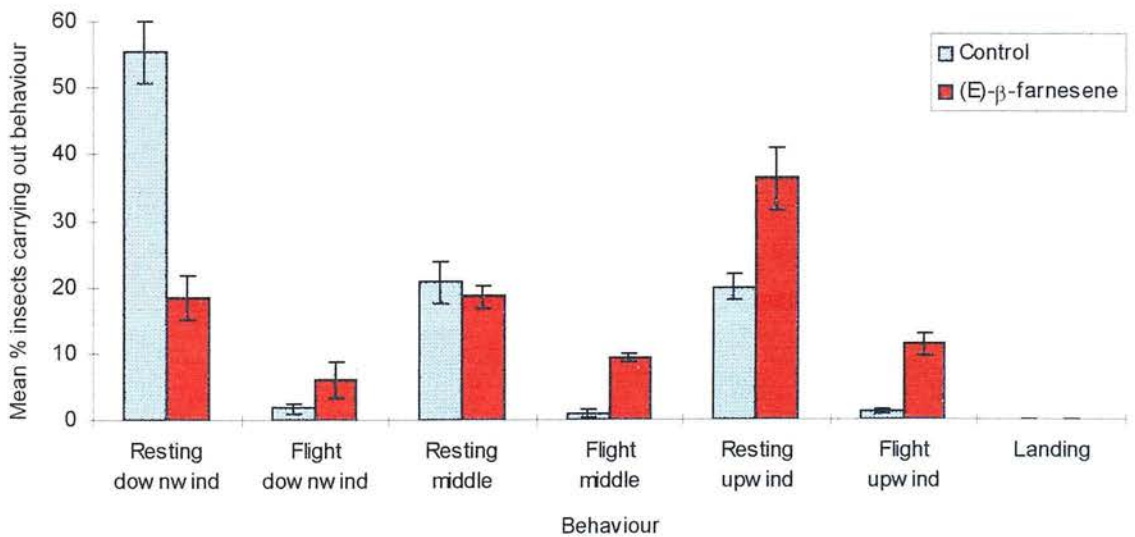


Fig 6.5 Mean % of female *E. balteatus* (\pm SE) carrying out specified behaviour in the wind tunnel with (E)-β-farnesene as an odour source (sample size=3)

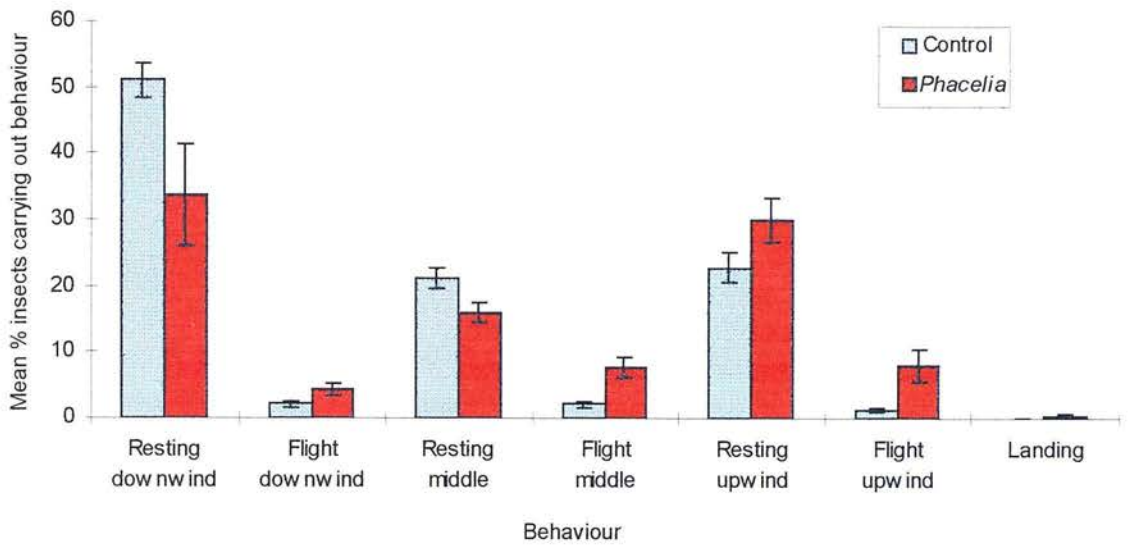


Fig 6.6 Mean % of male *E. balteatus* (\pm SE) carrying out specified behaviour in the wind tunnel with *Phacelia* flower extract as an odour source (sample size=3)

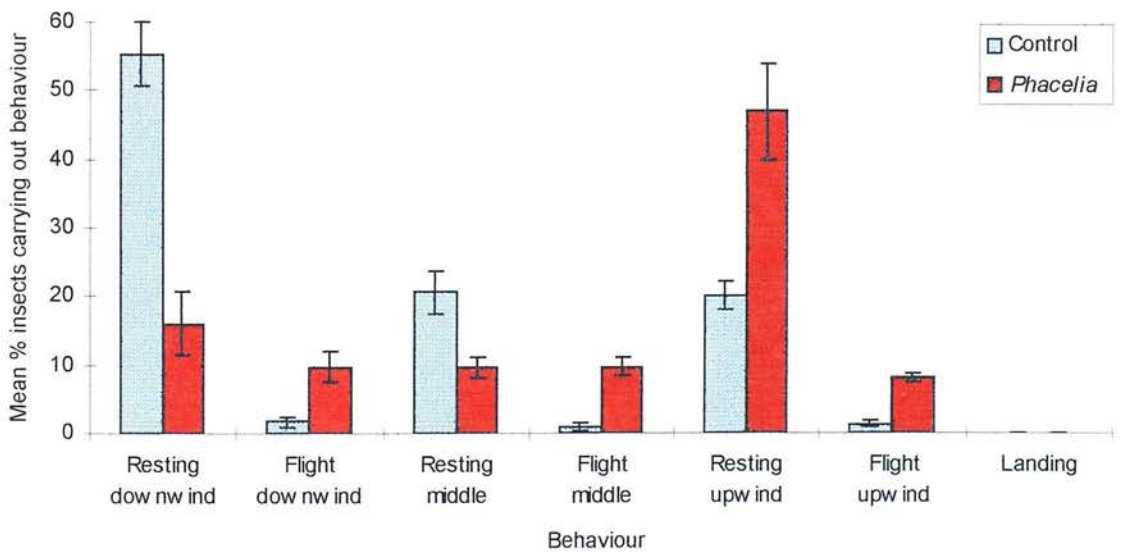


Fig 6.7 Mean % of female *E. balteatus* (\pm SE) carrying out specified behaviour in the wind tunnel with *Phacelia* flower extract as an odour source (sample size=3)



Fig 6.8 Mean % of male *E. balteatus* (\pm SE) carrying out specified behaviour in the wind tunnel with *Nepeta* extract as an odour source (sample size=3)

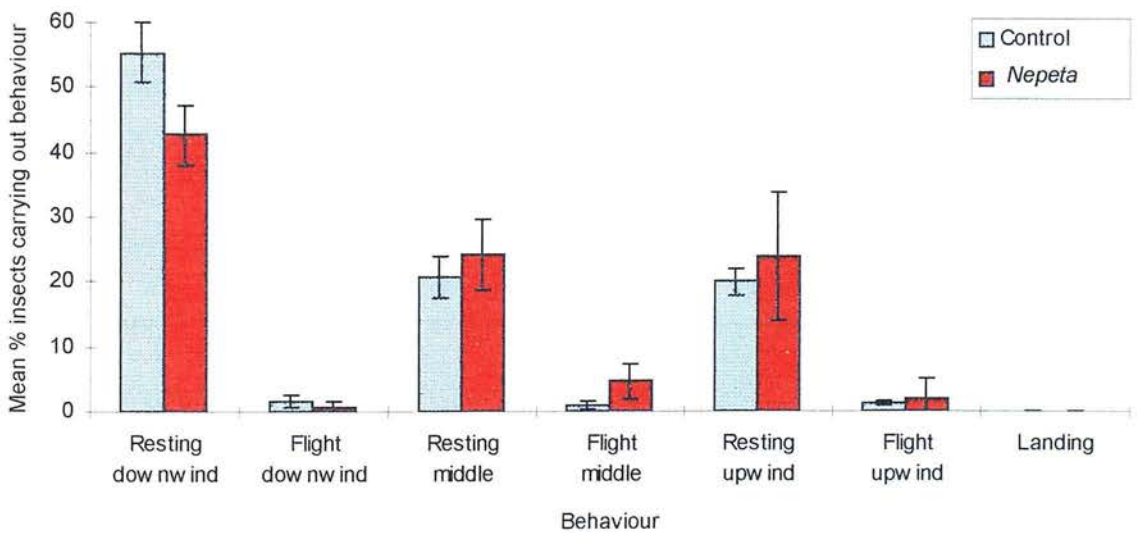


Fig 6.9 Mean % of female *E. balteatus* (\pm SE) carrying out specified behaviour in the wind tunnel with *Nepeta* extract as an odour source (sample size=3)

fewer hoverflies resting in the downwind section of the wind tunnel and with most treatments an increased number of hoverflies resting in the upwind section relative to the control (Figs. 6.2-7), except for the response of male *E. balteatus* to acetaldehyde or (E)- β -farnesene. There were also a significantly higher percentage of hoverflies undergoing flight behaviour throughout the wind tunnel. However, only female *E. balteatus* demonstrated a significant change in behaviour in response to *Nepeta* extract ($P < 0.05$, Fig. 6.9). Males did not show any significant behavioural change to this compound ($P > 0.05$, Fig. 6.8). P values for the behavioural responses of *E. balteatus* are shown in Appendix 2, Table 10.5.

6.4 Discussion

The results indicate that both male and female *E. balteatus* can detect and orient towards volatile semiochemicals from a distance of at least 1.75m from the odour source. Attractive semiochemicals include those associated with sources of adult food (*Phacelia*), and those associated with aphid prey for the aphidophagous larval stage; aphid alarm pheromone ((E)- β -farnesene) and aphid honeydew (acetaldehyde). The increased percentage of adult hoverflies in the upwind section of the wind tunnel in response to acetaldehyde, *Phacelia* and (E)- β -farnesene is indicative of odour-mediated upwind anemotaxis (Kennedy, 1977), the insects fly upwind following an odour plume to an odour source. Also with these semiochemicals there was a greater percentage of hoverflies undertaking flight behaviour in all sections of the wind tunnel.

Other dipterans have been shown to carry out positive anaemotaxis in the wind tunnel response to plant produced volatiles arising from the a food source. In the wind tunnel starved Mediterranean fruit flies, *Ceratitis capitata* Weid, were shown to respond and orientate to odours arising from coffee fruit (Prokopy *et al.*, 1998), a food source for the adult flies. *Phacelia tanacetifolia* provides a carbohydrate source for both male and female *E. balteatus* (Cowgill *et al.*, 1993a), therefore the hoverflies in the wind tunnel may be using the *Phacelia* extract volatiles as cues for food location.

Similarly acetaldehyde which initiates a behavioural response in both sexes of *E. balteatus* in the wind tunnel may be used by the hoverflies as a food location cue, as aphid honeydews provide a secondary carbohydrate source for both male and female *E. balteatus* (Gilbert, 1993). However in previous wind tunnel studies of *C. capitata*, well fed females were shown to increase their response to certain components of the coffee fruit volatile mixture, indicating that these components were not acting as food location cues but as oviposition cues (Prokopy *et al.*, 1998). Therefore while acetaldehyde is a food location cue for male *E. balteatus*, it may also act as an oviposition stimulant for gravid females.

Wind tunnel assays on several species of parasitoid have demonstrated that they orientate to semiochemical cues arising directly from their prey species (Ma *et al.*, 1992; Agelopoulos *et al.*, 1995; Zanen & Cardé, 1996). These semiochemicals acted as oviposition cues for gravid female parasitoids and initiated orientation in the wind tunnel. (E)- β -farnesene, the active component of the aphid alarm pheromone (Dawson *et al.*, 1983), caused orientation of *E. balteatus* in the wind tunnel. While

this may be a food location cue in male *E. balteatus*, enabling them to locate aphid colonies and therefore honeydew, in gravid females it may also act as an oviposition stimulant enabling them to locate aphid colonies not only for food, but for suitable oviposition sites.

Male and female *E. balteatus* demonstrated different responses to *Nepeta* extract in the wind tunnel, females were highly attracted to the extract, while males demonstrated no response. The differential response of the sexes to volatiles in the wind tunnels supports the findings of McDonald & Borden (1997), who found that in the onion maggot, *Delia antiqua* Meigen, a fly with herbivorous larvae, that although the adults responded to host plant volatiles in a wind tunnel, the response was affected by differences in age, sex, mated status and ovarian development. The females used the volatiles from onions as an oviposition cue and therefore showed a large response to onion odours in the wind tunnel. However male response to onion odours alone in the wind tunnel was extremely limited, but the response was increased if females were present. This suggest that for male onion maggots the volatiles from onions act as a limited mate finding cue (McDonald & Borden, 1997). This may be the case with *Nepeta* extract for *E. balteatus*, *Nepeta* may act as an oviposition cue for female *E. balteatus*. However, *Nepeta* extract is probably not a strong cue for males as it contains components of the aphid sex pheromone which is only produced at the end of summer, when most male *E. balteatus* are migrating to the continent during which time they neither feed or mate (Gilbert, 1993).

6.5 Summary

- Male and female *E. balteatus* exhibited a behavioural response to acetaldehyde, (E)- β -farnesene and *Phacelia* flower extract.
- Only female *E. balteatus* demonstrated a behavioural response to *Nepeta* extract.
- The behavioural response was indicative of odour mediated upwind anemotaxis.

Chapter 7

Field season 1996 and 1997

Chapter 7. Field season 1996 and 1997

7.1 Introduction

Each year aphids cause a significant amount of economic damage to arable crops around the world (Buntin, 1995; Kelm and Godomski, 1995). The fecundity and movements of aphid species can also be very high, and it has been reported that one aphid species *T. trifolii* was able to spread over an area of 50,000 Km² in less than a year (Hofsvang, 1990). There is also increasing incidence of pesticide resistance amongst pest populations, as well as problems with the inappropriate redistribution of pesticides in the environment (Pickett, 1995).

Predators usually have a high fecundity (Hofsvang 1990), and a very large potential to control pest populations (Singh & Singh, 1994a; 1994b). Introduction of coccinellids in Egyptian wheat fields caused a 46-49% increase in the yield. In the United States introduction of an exotic Asian ladybird *Harmonia axyridis* led to a significant reduction in the numbers of pecan growers who had to spray insecticides to control the pecan aphid, *Melanocallis caryaefoliae*, in the areas where it was introduced (Bauer, 1995).

However, pesticide treatments and fragmentation of agricultural habitats which removes overwintering habitats and other non-prey resources, led to cases of local extinction of predators from agricultural land (Jepson, 1984). In a study of cereal fields in East Anglia spraying pesticides was shown to reduce both the total number

of individual Carabidae present, and the species diversity of carabids in the field (Hawthorne & Hassal, 1994).

Studies in the UK have revealed that the number of aphidophagous *E. balteatus* adults and eggs was significantly increased in unsprayed field headlands as compared to herbicide treated headlands (Cowgill, 1993).

Populations of predators are generally not evenly distributed throughout the crop. In the spring carabids migrate into the crop from the field margins where they overwinter, however, it takes time for them to penetrate through the entire crop. Early in the season the majority of predators are only to be found in the first 1-10m away from the field margin (Dennis, 1991), but if predators are to carry out effective control of pests, then they have to penetrate the crop early in the season before large pest populations can become established. *Phacelia* flower extracts, *Nepeta* leaf extract and (E)- β -farnesene have been shown to be attractive to both *E. balteatus* and *P. melanarius* in laboratory studies (Chapters 5 and 6).

The work outlined in this chapter aims to determine the effects of these compounds on predator numbers in the field, particularly on the number of carabid beetles present in the centre of the field.

Populations of ground dwelling invertebrates in the field can be monitored using several different techniques including suction sampling such as D-vacs, absolute sampling methods and pitfall trapping (Schotzko & O'Keefe, 1989).

Suction sampling involves the use of a large petrol powered vacuum, which can be used to suck any debris and invertebrates from the ground, and can be used to obtain samples of the entire population of invertebrates including both active and non active

individuals. However if there is extensive vegetation cover, such as in a cereal field, then the invertebrates may take refuge under the vegetation, to be effective plants must be removed from the area or only a single individual plant can be sampled at once (Schotzko & O'Keefe, 1989). In a commercial cereal field, removing large areas of the crop is unfeasible.

Absolute sampling methods provide a good estimation of absolute population densities, however, they rely on the removal of large areas of the crop together with the first 30cm of top soil which are then thoroughly rinsed in running water to wash out the invertebrates (Schotzko & O'Keefe 1989). However this method would be too destructive in a commercial cereal field, and would mean that only one sample could be obtained before the habitat is destroyed.

Pitfall trapping involves the placement of a container into the ground that ground dwelling invertebrates fall into and cannot then get out of (Van den Berghe, 1992). However, pitfall trapping will not give accurate information about population densities, because it relies on the activity of individual beetles, which in turn is dependent on a number of diverse factors such as time of sampling, climatic conditions, species, physiological condition and even sex of the individual invertebrate (Luff, 1987). Therefore, pitfall trapping frequently leads to a large underestimation of the population densities of invertebrates in an area, because there may be large numbers of invertebrates in the area but they may be inactive (Luff, 1987), such as in the summer developmental diapause of *N. brevicollis*. However, pitfall trapping can be used to provide an accurate measure of the "activity-density" of invertebrate populations (Luff, 1987).

Pitfall trapping was employed in the present study to monitor populations of ground dwelling carabids, as it's the least invasive of any sampling method causing minimum disturbance to the habitat, and as inactive invertebrates generally do not feed, they cannot have a large impact on prey populations (Thiele, 1977). Pitfall trapping does provide a good indication of the number of active beetles in the area, therefore is an accurate measure of the number of beetles foraging for prey and likely to have the greatest impact on pest populations.

Sticky traps were not used to monitor the populations of flying insects or insects in the crop canopy as the majority of sticky traps are coloured. Many insects demonstrate differential attraction to coloured traps (Broumas & Haniotakis, 1994), so it may be the trap that attracts the insects into the area rather than the semiochemicals. Sweep netting is the most commonly used method of sampling for invertebrates in the crop canopy, as it's a quick technique and can cover large areas of the crop, however this can lead to an overestimation of population densities (Schotzko & O'Keefe, 1989). In the present study a combination of sweepnet samples and direct observation was used to monitor insect populations.

7.2 Materials and methods

7.2.1 Field studies 1996

Field trials took place between April and August 1996. The field season of 1996 was a pilot study where all the chemicals identified as possible attractants in the electrophysiological and behavioural assays (Chapter 4,5 and 6) were tested for their

attraction to predators under field conditions. These chemicals included methanol controls, (E)- β -farnesene, *Nepeta* extract and *Phacelia* flower extract, artificial honeydew, L-tryptophan, and Envirofeast sprays.

The treatments were tested in two fields of crops, a winter wheat field, cv Riband, at Boghall farm (Midlothian, UK, OS map 66, 653242) and a spring barley field, cv Prisma, at Glencarse farm (Midlothian UK, OS map 66, 638232). Two different crops were assessed to ensure that the treatments would be effective in different crops, however spring barley was chosen as the second crop over oilseed rape due to the practical difficulties in getting to the centre of a crop of oilseed rape to dig pitfall traps and to carrying out the weekly sampling without causing significant damage to the crop. Previous crops in the field included winter barley in the winter wheat field and potatoes in the spring barley field, and this may have had a slight effect on the fauna present in the fields at the beginning of the field season.

Although artificial honeydew or L-tryptophan did not initiate a response in predators during electrophysiological or behavioural trial, when used in a field situation they have been shown to cause a significant increase in the number of beneficial insects in the area (Ben Saad & Bishop, 1976; McEwen *et al.*, 1994). This indicates that although these compounds are not volatile attractants they may act as contact arrestants, initiating a short range searching behaviour in the predator when sensilla on other parts of the predators body, such as on the mouthparts, tarsi or ovipositor (Frazer, 1976) come into contact with the compound. This could be used in conjunction with long range volatile attractants, with the volatile compounds first attracting insects into the area then the contact chemicals acting to keep them in the

area. The best method of application of the semiochemicals was also examined, with semiochemicals used in dispensers at ground level and at canopy level.

The spring barley field was slightly smaller than the winter wheat field (10.01 ha spring barley, 11.28 ha winter wheat), therefore there was not enough space for all of the trials to be carried in spring barley. In this case the L-tryptophan spray was not used and artificial honeydew was substituted for Envirofeast™ (Rhône Poulenc Agrochemicals Ltd, Australia). Envirofeast™ is a commercially produced honeydew substitute undergoing trials in Australia, preliminary results suggest that this compound causes a significant increase in the number of predators present in cotton crops (Mensah, 1997), However the producers required trials in other crops such as cereals. This also provided an opportunity for the possible attractants identified in previous trials to be assessed alongside a commercially produced compound for pest control.

Acetaldehyde was not included in these trials as there was limited space available for test compounds in the field trials and both *P. melanarius* and *N. brevicollis* exhibited no response to acetaldehyde and the behavioural trials involving *E. balteatus* had not been completed. However this does provide possibilities for field trials in the future.

The fields were divided into 27 50x50m plots, a distance of 50m being recommended by manufacturers of commercial semiochemicals to ensure that there is no interaction between different semiochemical dispensers in the plots. At the centre of each plot, a 10m x 10m experimental area was marked off using canes. Five covered dry pitfall traps were placed at random in the experimental area, the

covers on the traps allowed access to the traps by beetles and other insects while preventing small amphibians and small mammals from falling in. The beetles trapped in the pitfalls were needed for use in enzyme linked immunosorbant assays (ELISAs), therefore the traps did not contain a preservative as this may produce false results in the assays. Therefore the beetles were kept live in the traps and when the traps were emptied, they were transported back to the laboratory as quickly as possible, where they were preserved by freezing. These measures were to reduce predation and cannibalism in the traps, however a limited amount of cannibalism probably still took place within the traps.

Three replicates of each of the treatments were assigned at random to the plots in the field (Fig. 7.1). For the treatment requiring semiochemical dispensers, the dispensers were placed in the centre of the experimental areas. The dispenser consisted of a plastic universal bottle (50ml, 4cm diameter x 6cm) with screw thread metal cap, through which a wick of woven cotton (0.2cm diameter) protruded 2cm. A hood made from finger bandage tied at the top with cotton thread covered the wick. This protected the wick and prevented the ingress of rainwater into the dispenser, which would dilute the semiochemical (Fig. 7.2).

At weekly intervals, 50ml of fresh semiochemical was placed in the dispensers (1/10 dilution (E)- β -farnesene, fully concentrated plant extracts), and 250ml of artificial honeydew, L-tryptophan or Envirofeast™ (25g Envirofeast™ powder) in 250ml distilled water, were sprayed over the experimental area using a 500ml houseplant sprayer (Klondyke Garden Centres, Stirling, UK). The pitfall traps were emptied and 10 sweepnet samples were taken at random from the experimental area. Ten plants

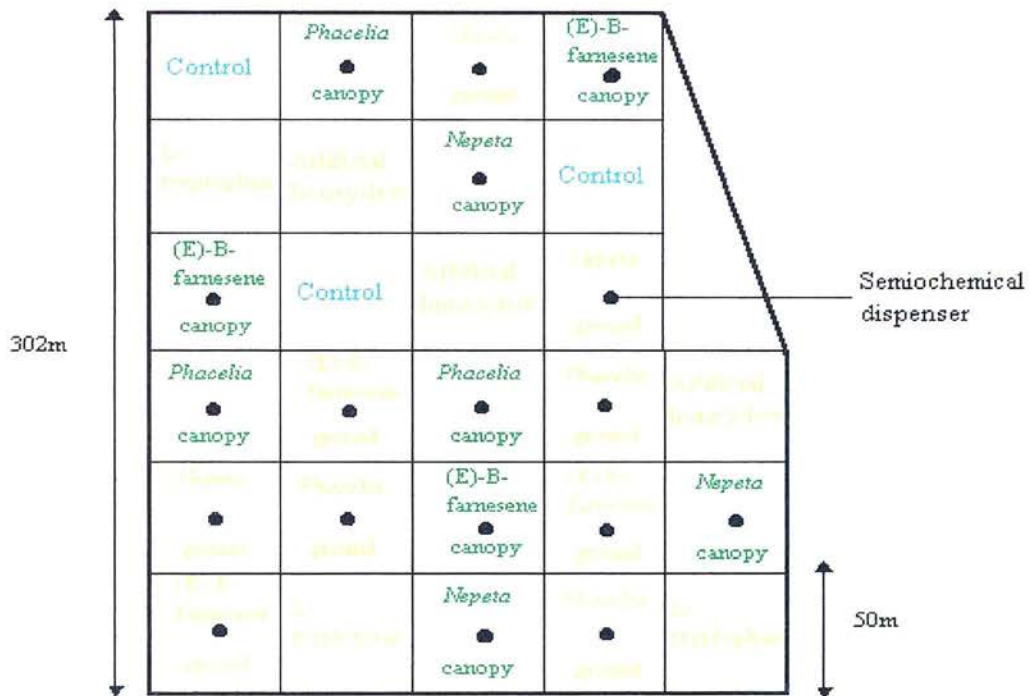


Fig. 7.1 Field layout in 1996.

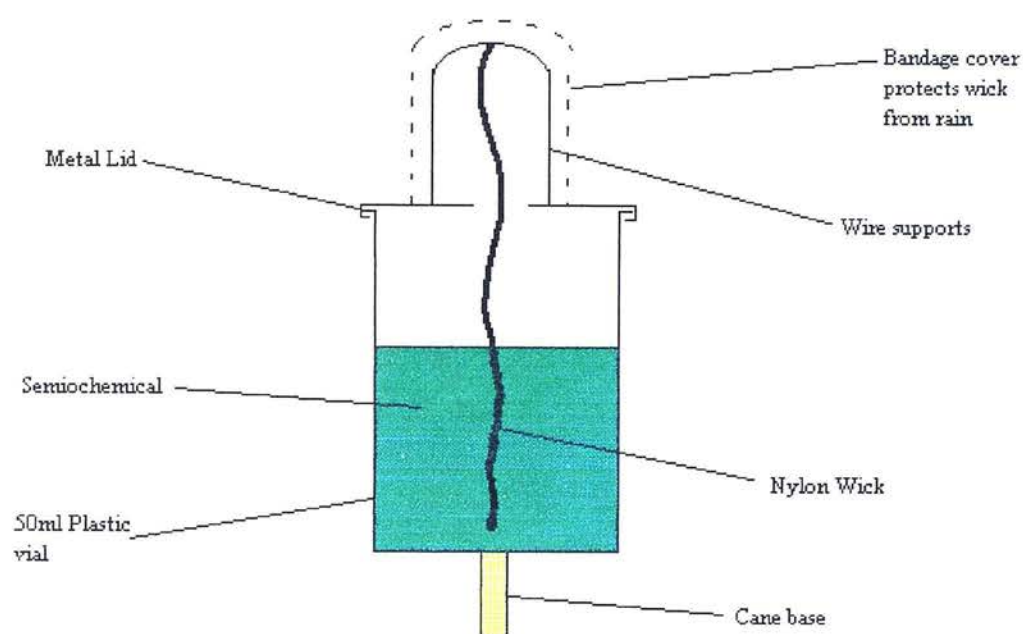


Fig. 7.2 The semiochemical dispenser

were examined at random for the presence of pest species, mummies, or the larval or adult stages of beneficial insects.

The data, expressed as the mean number of carabids or aphids per treatment, were analysed by a single factor analysis of variance. The experimental design and statistical analysis would not detect less than a two fold increase in predator numbers, however in a commercial situation if a treatment does not cause at least a two fold increase in predator numbers then it probably will not have a large enough impact on pest populations to be feasible.

7.2.2 Field studies 1997

From the previous years studies, Coccinellids, and Syrphids were not present within the crop in significant numbers, so the 1997 study concentrated on the effects of semiochemicals on the behaviour of Carabids within the arable field system, as these seem to constitute the major group of predators within the fields under study.

One of the fields was located at Boghall Farm, Midlothian (UK), while the other 2 were located at Braid Hills Farm (Midlothian, UK, Os map 66, 702262). The field trials were carried out between April to August 1997. The three winter wheat fields (cv. Riband) selected had an approximate area of 10.04, 10.21 and 11.03 hectares respectively. The three fields are located in the same proximity, therefore were subject to the same prevailing environmental conditions.

The semiochemicals used in these trials were identified as being the most attractive to beneficial arthropod predators from the previous years study. These included methanol (control) in ground level dispensers, (E)- β -farnesene in canopy level

dispensers, *Nepeta* extract in ground level dispensers, *Phacelia* flower extract in ground level dispensers, and Envirofeast™ spray with a distilled water spray control.

The fields were divided into 6 10 x 100m strips spaced at least 50m apart to avoid any interaction between the semiochemicals in each strip. To give a measure of distance to the field boundary, only one of the short (10m) edges was in contact with a field boundary. Each of the other edges was at least 100m away from the parallel field boundary (Fig. 7.3). Three 10 x 10m sampling plots were marked out in each strip from 0-10m, 45-55m, and 90-100m from the field boundary, using canes. Five dry covered pitfall traps were placed at random in each of the sampling areas.

Test compounds and controls were randomly assigned to one of the strips in each field. Wicked semiochemical dispensers were placed in a row down the centre of each strip every 20m and 50ml of the methanol control, plant extract or 10^{-1} dilution of (E)- β -farnesene was placed in each dispenser. The two remaining strips in each field were sprayed with either 2.5 litres of Envirofeast™ (150g in 2.5L distilled water) or by 2.5L of distilled water control. Once per week the semiochemicals in the dispensers were refreshed and the spray treatments re-applied. The pitfall traps were emptied and 10 sweep net samples were taken at random, Ten plants chosen at random were visually examined for the presence of pest species or mummies, or for larval and adult stages of beneficial predators.

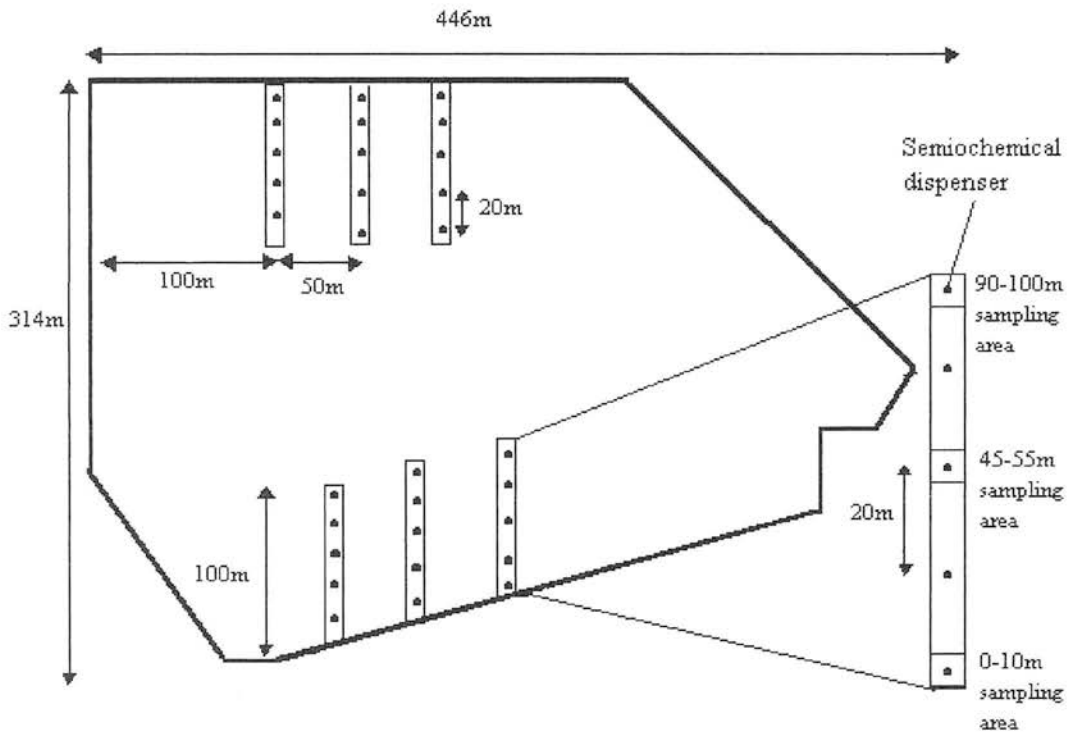


Fig. 7.3 The layout of Fields 1997

7.2.3 Statistical analysis.

The number of active beetles and aphids per plot was analysed by a generalised linear model (Edgington & Gore, 1986; Edgington, 1995) using the Genstat 5 package (Copyright 1995, Lawes Agricultural Trust- Rothamsted Experimental Station, Table 7.1). The model assumed that the data had a negative binomial distribution and therefore used a log-ratio link function. The negative binomial distribution fitted the experimental data much better than a Poisson distribution with a log link function, as demonstrated by the residual mean deviance. The closer the residual mean deviance is to 1 then the better the fit of the model. A negative binomial distribution indicates that the beetles occur in a clumped distribution with either a high number of active beetles or very few active beetles in the plot, with little in between these extremes. Although the negative binomial distribution is the best estimate of what is happening in the field and fits the data better than other distributions, it was not a perfect fit shown by the residual mean deviance of 1.349 rather than 1, this may lead to a few anomalous points in the predictions.

The model fitted field which was regarded as a block factor and treatment, distance and week which were regarded as treatment factors. A series of progressively more complicated models were fitted to the negative binomial distribution of beetle numbers and for each model mean deviances were calculated. Mean deviances for each progressive model were compared to the residual mean deviance to give a variance ratio test. Predicted means were calculated from the parameters of the model however standard errors were increased to compensate for a mean deviance of 1.349 rather than 1.

7.3 Results

7.3.1 1996

7.3.1.1 Winter wheat

For all treatments there was a significant difference in the number of beetles trapped per week (artificial honeydew $P < 0.05$, L-tryptophan $P < 0.01$, (E)- β -farnesene in ground level dispensers $P < 0.01$, (E)- β -farnesene in canopy level dispensers $P < 0.001$, *Nepeta* extract in ground level dispensers $P < 0.001$, *Nepeta* extract in canopy level dispensers $P < 0.001$, *Phacelia* extract in ground level dispensers $P < 0.001$, and *Phacelia* extract in canopy level dispensers $P < 0.001$).

There were two main peaks of carabid activity, with a large number of carabids being trapped from late April to early June, then with a decline in beetle numbers. The next peak in beetle activity occurs from early July until the beginning of August (Figs. 7.4-8).

In winter wheat there was no significant difference in the numbers of beetles trapped in the artificial honeydew and L-tryptophan plots relative to the control plots ($P > 0.05$, Figs. 7.4 and 7.5).

With the (E)- β -farnesene treatment, there was an overall significant increase in the number of beetles trapped in the plot with the canopy level dispensers relative to the controls throughout the field season ($P < 0.01$, Fig. 7.6), however there was no significant increase in the number of beetles trapped with the ground level dispensers relative to the controls ($P > 0.05$).

Table 7.1 Randomised linear model for Field data analysis

<pre> job open name='data';channel=2 factor [labels=!t('1.-10','45-55','90-100')] sdist factor sweek,sfield read [channel=2] sweek,sdist,sfield,v[1...6];frep=lev,lab,lev,6(*) tabu [print=counts;class=sweek] & [class=sdist] & [class=sfield] factor [nvalues=702;labels=!t(control,Spray_Co,Phacelia,Nepeta,'(E)-B-F','\En feast); values=117(1...6) treat factor [nvalues=702;levels=13] week factor [nvalues=702;levels=3] field factor [nvalues=702;levels=3;labels=!t('1.-10','45-55','90-100')] dist equat old=!p((sweek)6);new=week & old=!p((sdist)6);new=dist & old=!p((sfield)6);new=field & old=v;new=nbeetles print week,dist,field,treat,nbeetles;dec=0;fieldw=8 </pre>	<p>Inputs data into model in the correct the form so subsequent analysis can take place.</p> <p>Tabulates and the data into week, distance and field and displays the tables</p> <p>Displays data for identification of incorrectly entered data.</p>
<pre> model [dist=neg;disp=*] nbeetles fit field+dist+week*treat predict [print=d,p,s] field & dist & week & treat & week,treat endjob </pre>	<p>Sets the parameters of the model and fits a series of progressively more complex factors.</p> <p>Generates and displays the predictions.</p>

With *Nepeta* extract and *Phacelia* flower extract in both canopy and ground level dispensers there was a significant increase in the overall mean number of beetles trapped throughout the season relative to the controls (Figs. 7.6 and 7.7). However, in both cases the overall increase was greater with the ground level dispensers ($P < 0.001$) than with the canopy level dispensers ($P = 0.02$ and $P = 0.003$ respectively). There was a significant decrease in aphid numbers with artificial honeydew and L-tryptophan treatments ($P < 0.001$, Figs. 7.9 and 7.10). With (E)- β -farnesene there was a significant fall in aphid numbers with both ground ($P < 0.001$, Fig. 7.11) and canopy level dispensers ($P < 0.01$).

With both *Nepeta* extract and *Phacelia* flower extract there was a significant decline in aphid numbers with both the ground ($P < 0.01$, Figs. 7.12 and 7.13) and canopy ($P < 0.01$) level dispensers.

7.3.1.2 Spring barley

There was no significant difference in the beetles trapped with Envirofeast™ spray relative to the controls ($P > 0.05$, Fig. 7.14). Significantly more beetles were trapped in the (E)- β -farnesene plots with canopy level dispensers ($P < 0.05$, Fig. 7.15), however, there was no significant difference with beetle numbers trapped in the plot with ground level dispensers relative to the controls ($P > 0.05$).

With both *Nepeta* extract and *Phacelia* flower extract there was a significant increase in the number of beetles trapped with ground level dispensers ($P < 0.05$ Figs. 7.16 and 7.17). With canopy level dispensers, although there was an increase in beetle numbers relative to the controls it was not significant ($P = 0.097$).

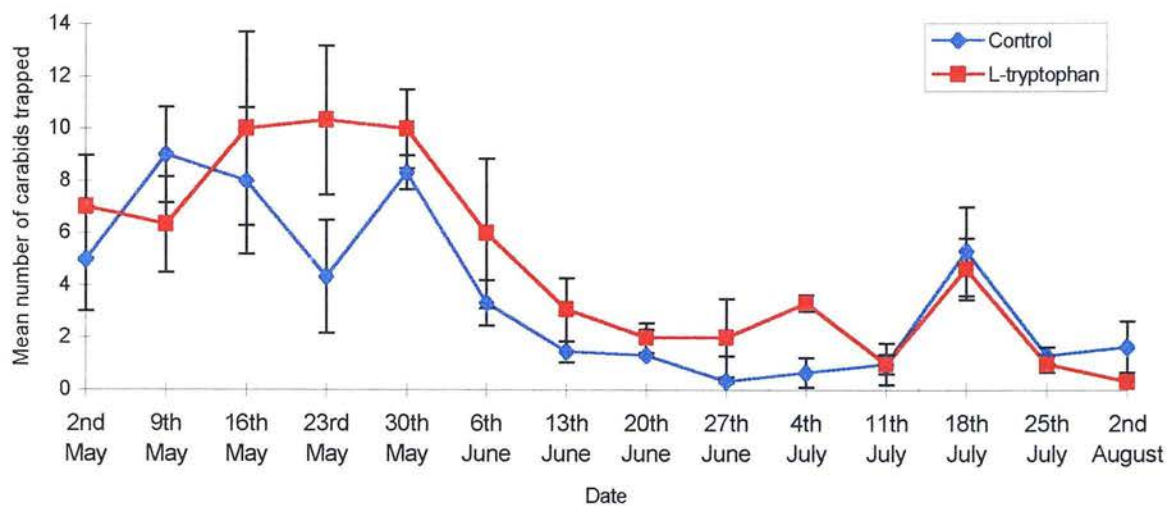


Fig. 7.4 Mean number of carabids (\pm SE) trapped in winter wheat in 1996 with artificial honeydew treatment (sample size=3).

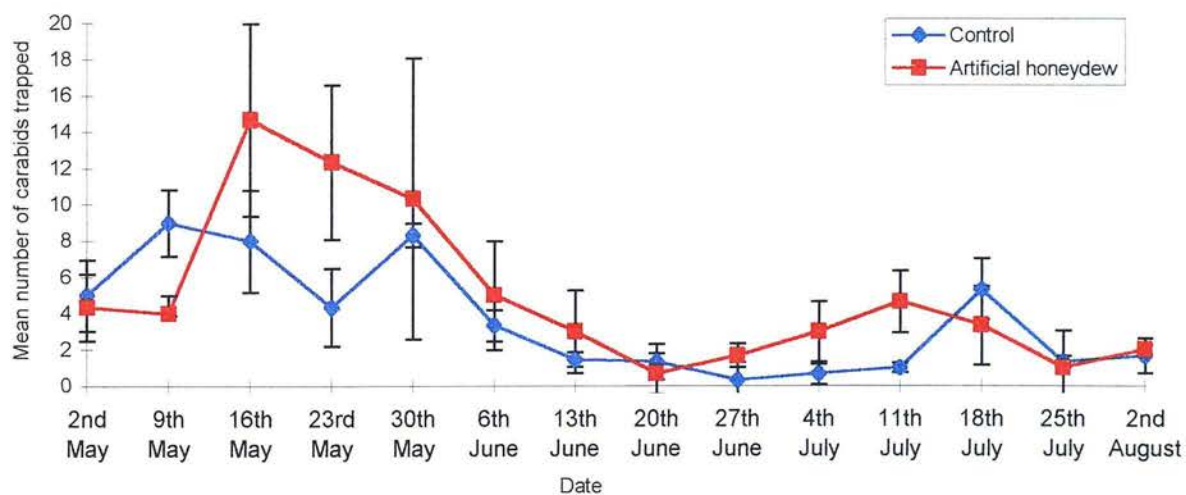


Fig. 7.5 Mean number of carabids (\pm SE) trapped in winter wheat in 1996 with L-tryptophan treatment (sample size=3).

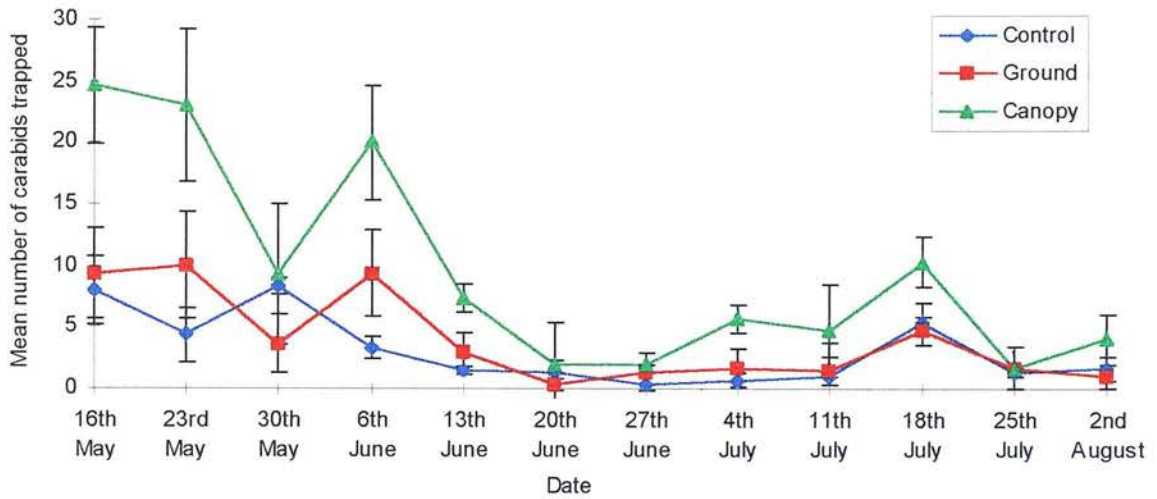


Fig. 7.6 Mean number of carabids (\pm SE) trapped in winter wheat in 1996 with (E)-β-farnesene treatment (sample size=3).

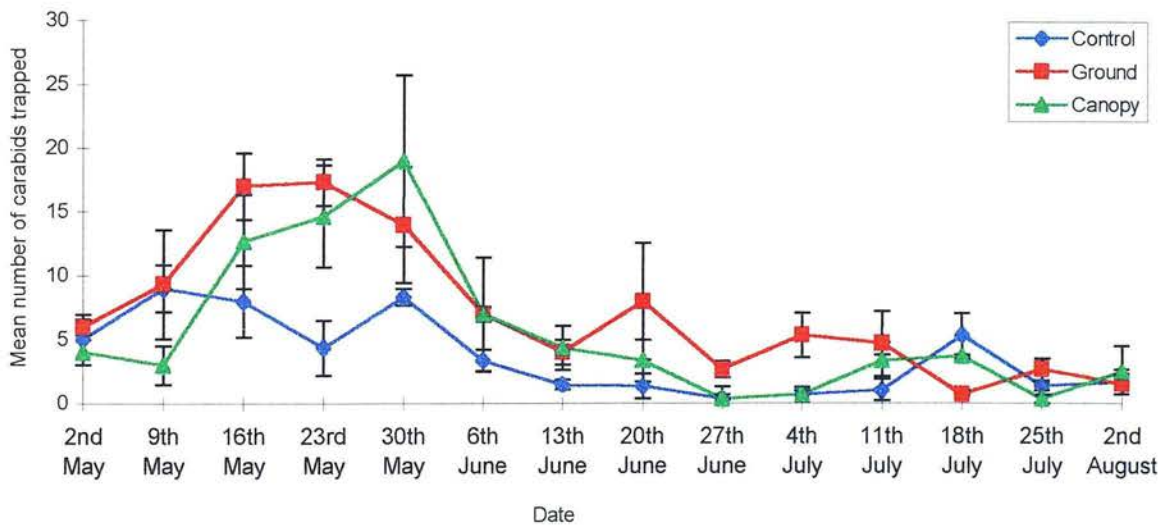


Fig. 7.7 Mean number of carabids (\pm SE) trapped in winter wheat in 1996 with *Nepeta* extract treatment (sample size=3).

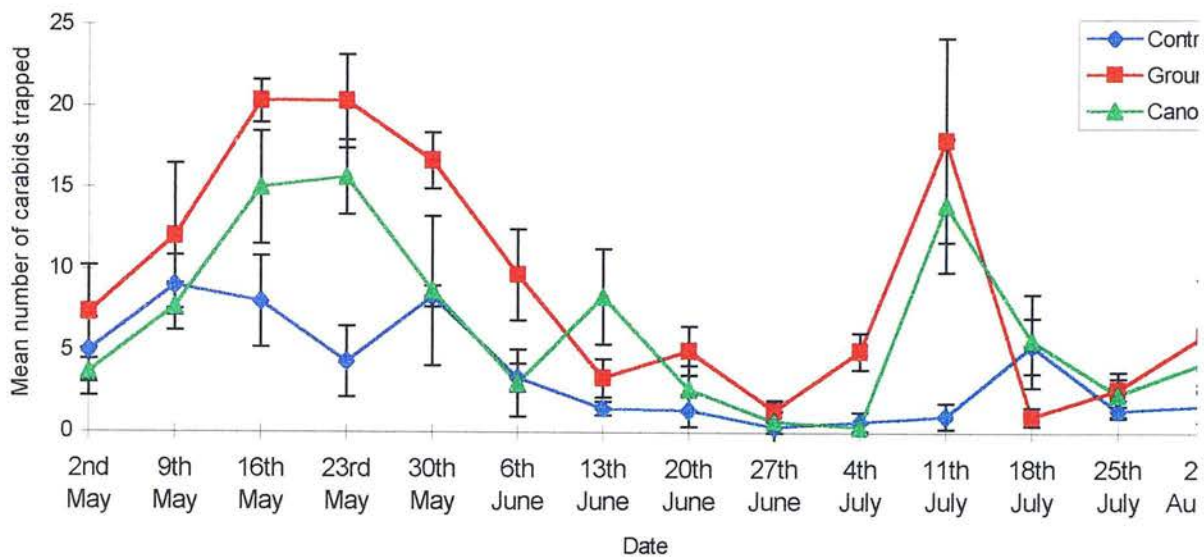


Fig. 7.8 Mean number of carabids (\pm SE) trapped in winter wheat in 1996 with *Phacelia* flower extract treatment (sample size=3).

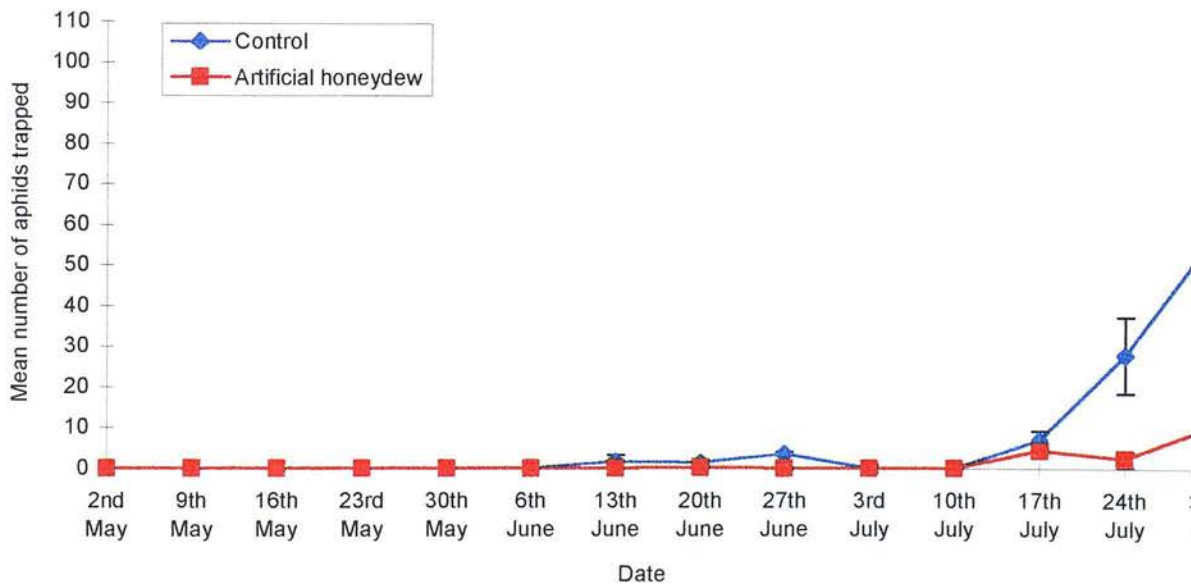


Fig. 7.9 Mean number of aphids (\pm SE) trapped in winter wheat in 1996 with artificial honeydew treatment (sample size=3).

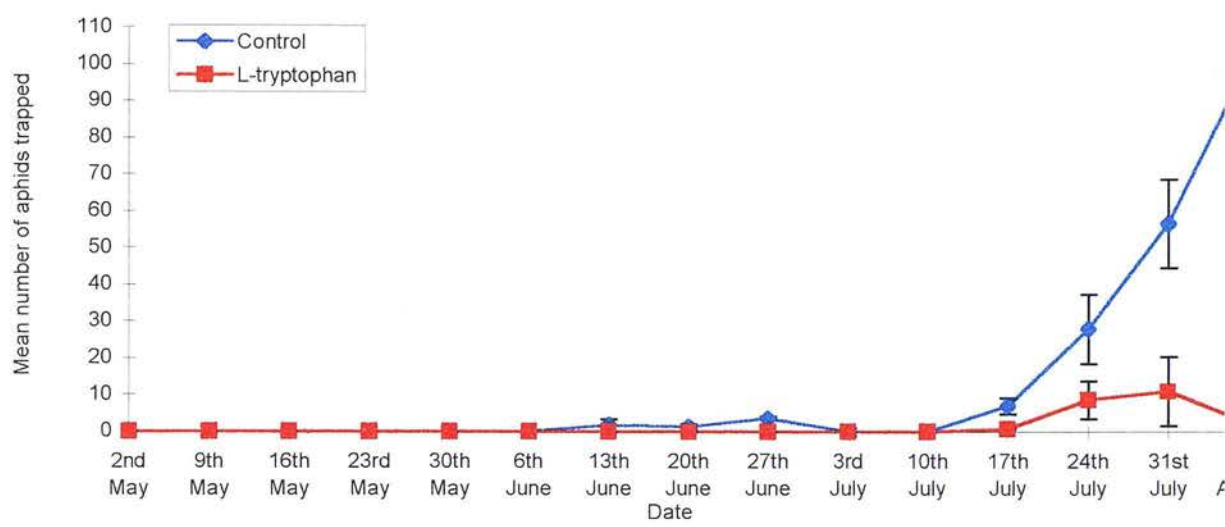


Fig. 7.10 Mean number of aphids (\pm SE) trapped in winter wheat in 1996 with L-tryptophan treatment (sample size=3).

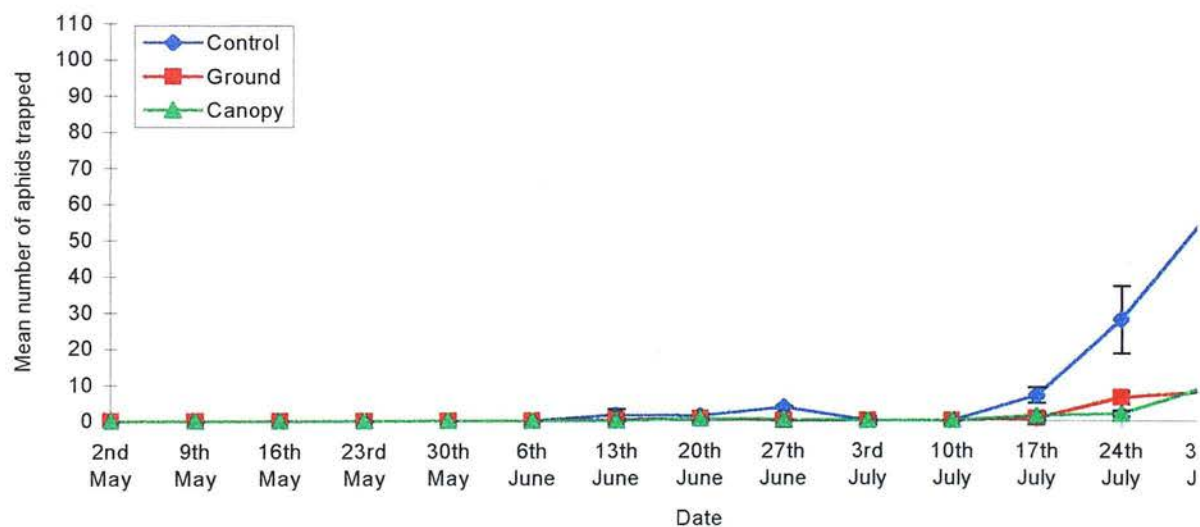


Fig. 7.11 Mean number of aphids (\pm SE) trapped in winter wheat in 1996 with (E)- β -farnesene treatment (sample size=3).

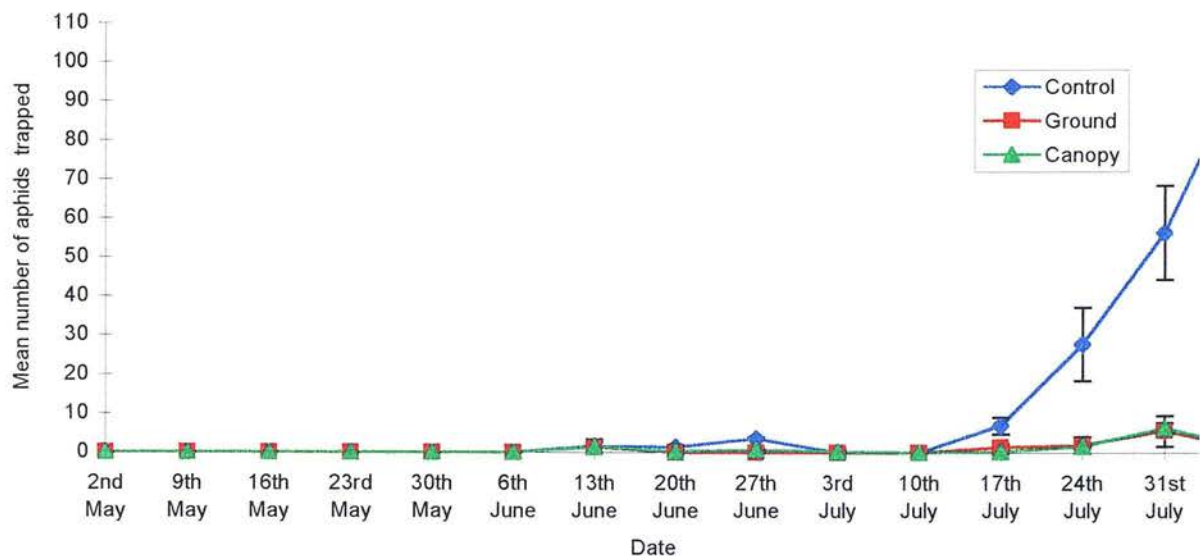


Fig. 7.12 Mean number of aphids (\pm SE) trapped in winter wheat in 1996 with *Nepeta* extract treatment (sample size=3).

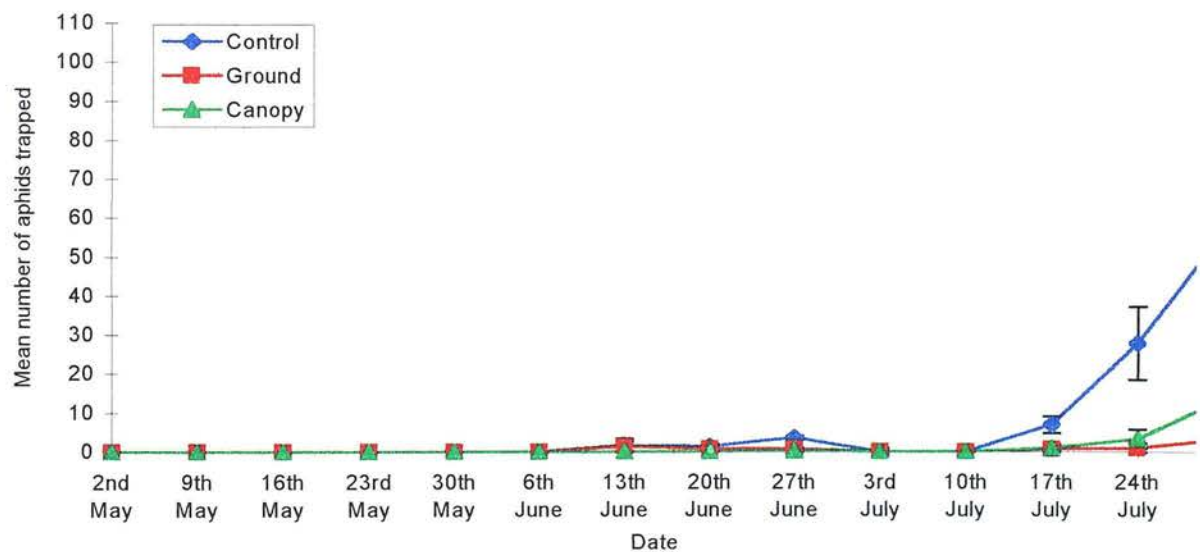


Fig. 7.13 Mean number of aphids (\pm SE) trapped in winter wheat in 1996 with *Phacelia* Flower extract treatment (sample size=3).

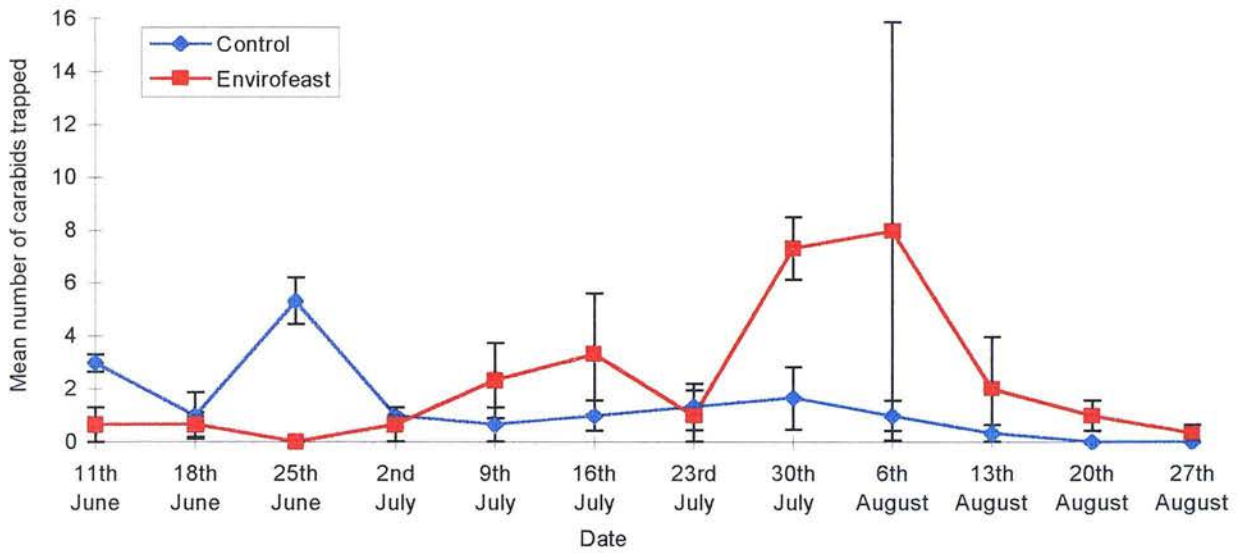


Fig. 7.14 Mean number of carabids (\pm SE) trapped in spring barley in 1996 with EnvirofeastTM treatment (sample size=3).

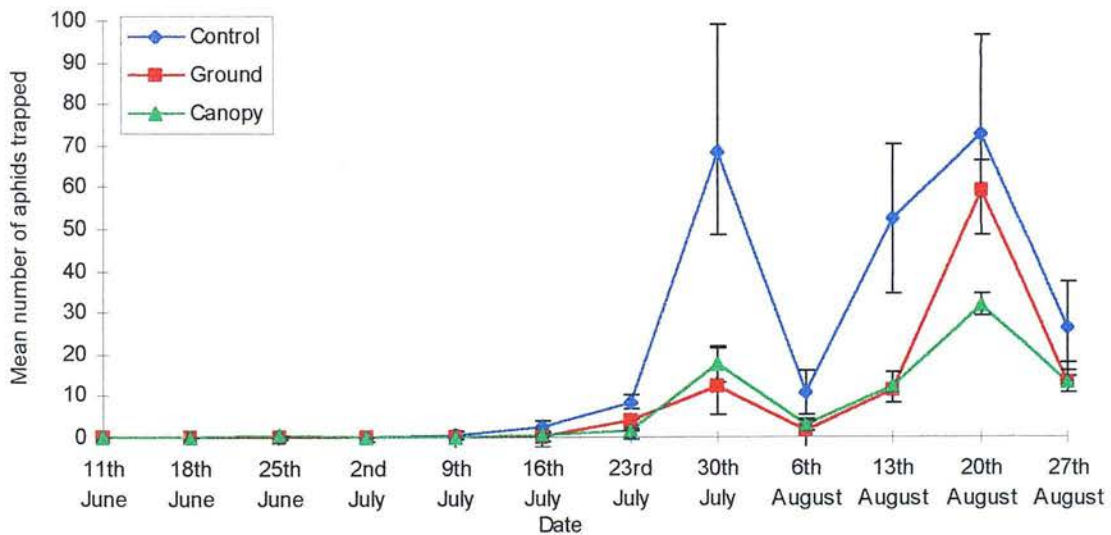


Fig. 7.15 Mean number of carabids (\pm SE) trapped in spring barley in 1996 with (E)- β -farnesene treatment (sample size=3).

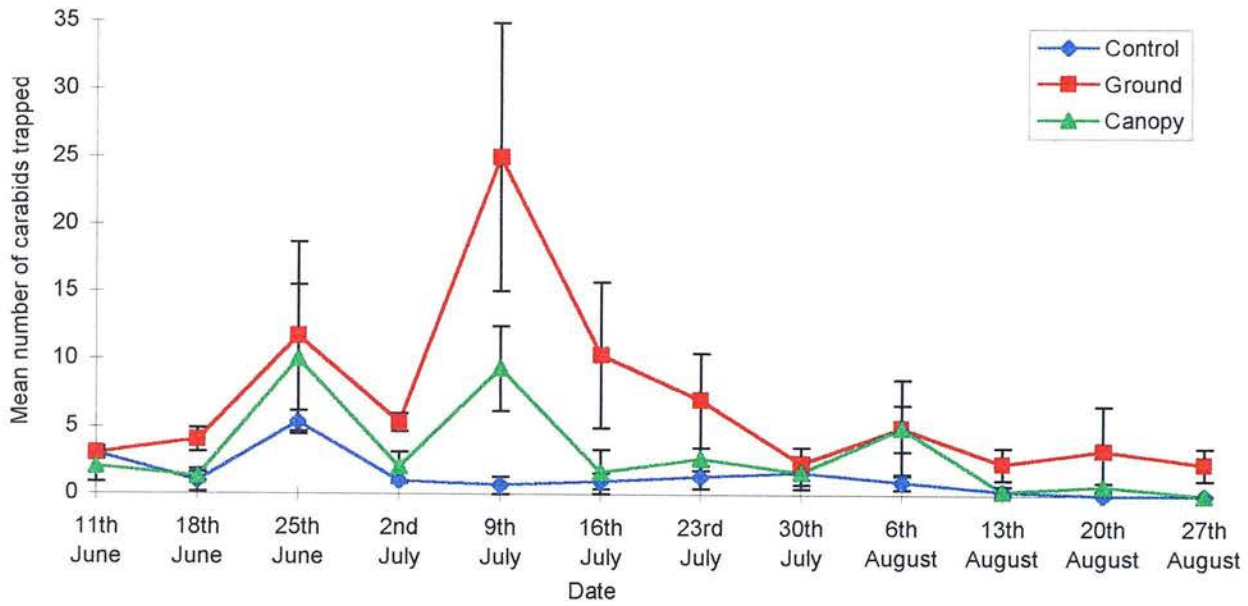


Fig. 7.16 Mean number of carabids (\pm SE) trapped in spring barley in 1996 with *Nepeta* extract treatment (sample size=3).

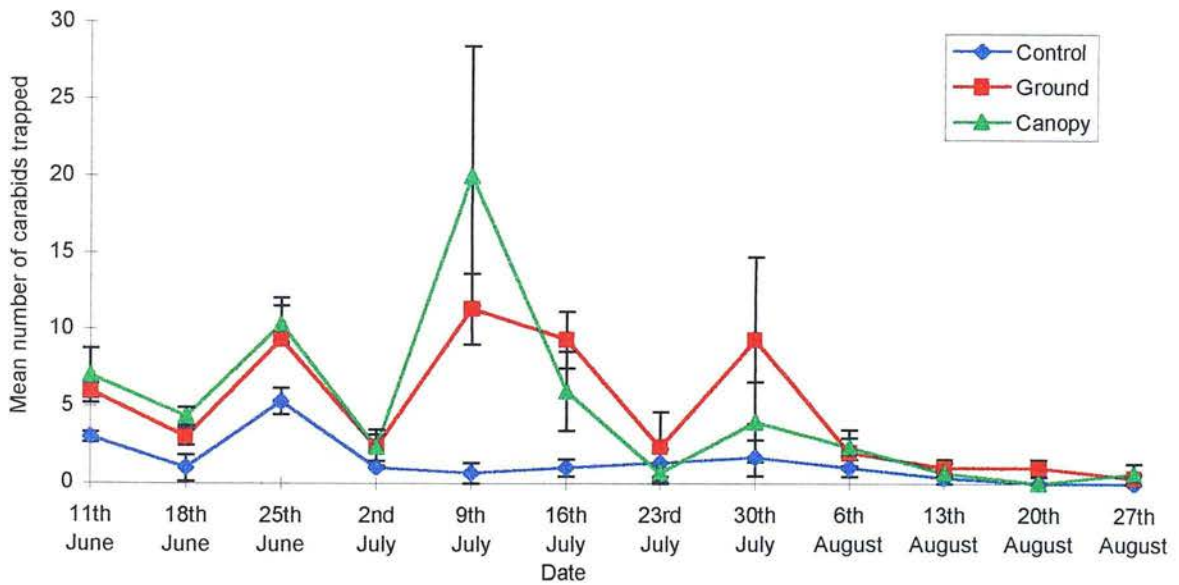


Fig. 7.17 Mean number of carabids (\pm SE) trapped in spring barley in 1996 with *Phacelia* flower extract treatment (sample size=3).

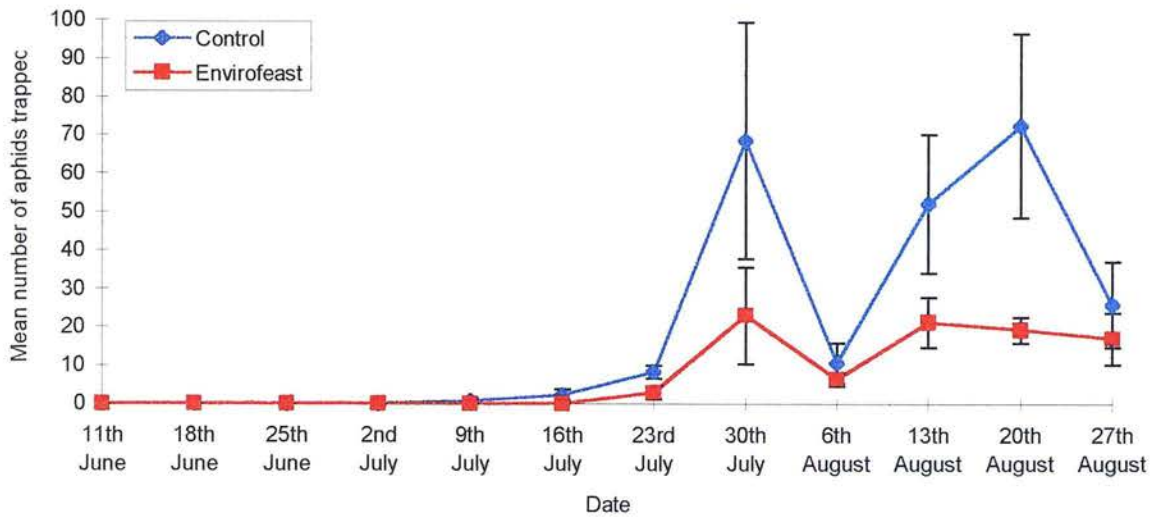


Fig. 7.18 Mean number of aphids (\pm SE) trapped in spring barley in 1996 with EnvirofeastTM treatment (sample size=3).

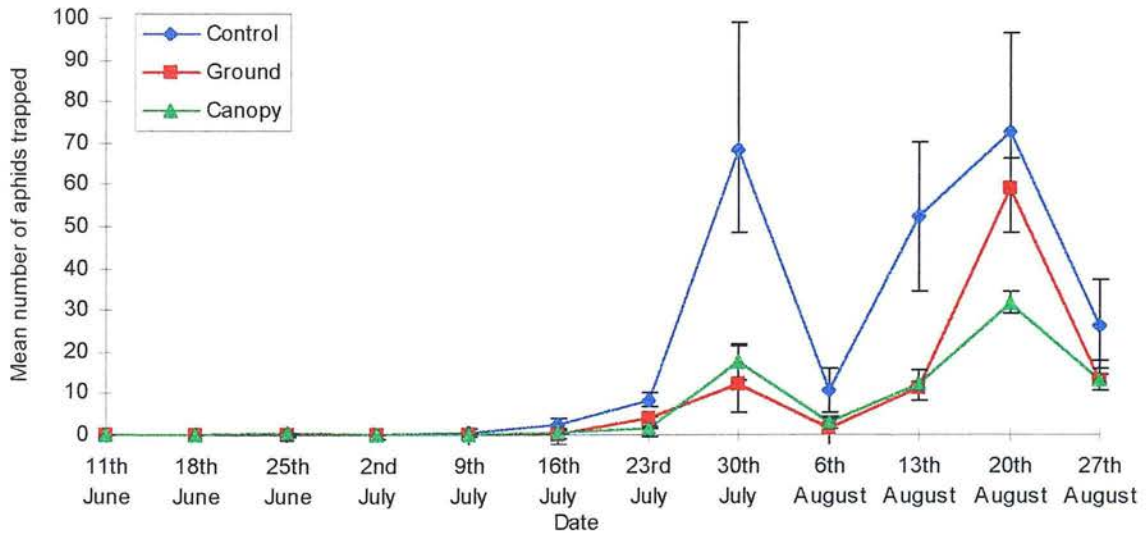


Fig. 7.19 Mean number of aphids (\pm SE) trapped in spring barley in 1996 with (E)- β -farnesene treatment (sample size=3).

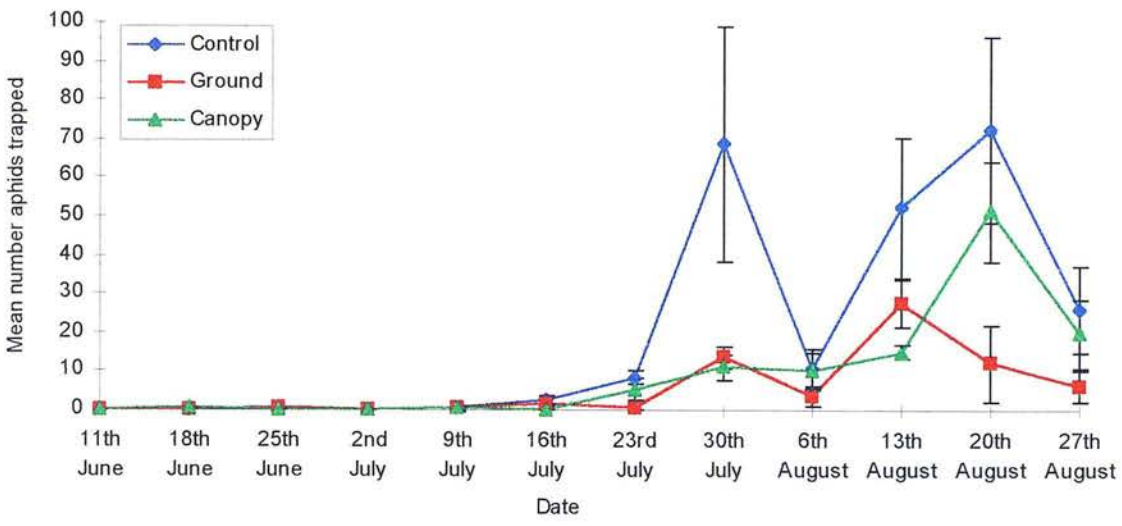


Fig. 7.20 Mean number of aphids (\pm SE) trapped in spring barley in 1996 with *Nepeta* extract treatment (sample size=3).

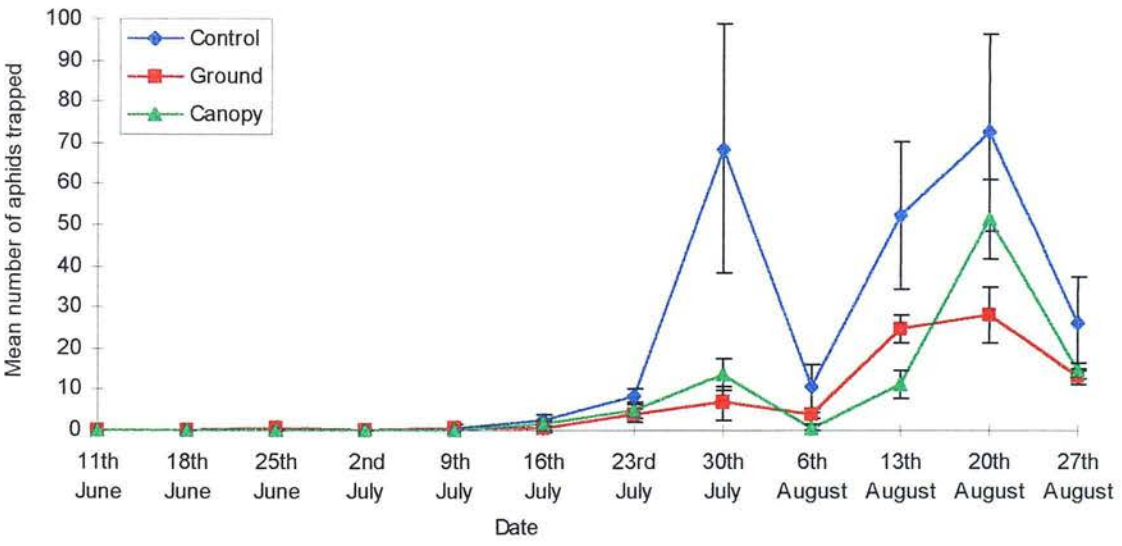


Fig. 7.21 Mean number of aphids (\pm SE) trapped in spring barley in 1996 with *Phacelia* flower extract treatment (sample size=3).

and $P=0.067$ respectively). Of the three compounds which led to an increase in carabid numbers the greatest increase occurred with *Phacelia* extract in ground level dispensers.

There was a significant decrease in aphid numbers in Envirofeast™ plots relative to the control plots ($P<0.05$, Fig. 7.18). With (E)- β -farnesene, *Nepeta* extract and *Phacelia* extract there was a significant decline in aphid numbers with both ground and canopy level dispensers. With (E)- β -farnesene the greatest decline occurred in canopy level dispensers ($P < 0.001$ canopy and $P < 0.01$ ground, Fig. 7.19).

Whereas with both *Nepeta* and *Phacelia* extracts a significant decline in aphid numbers occurred with the ground level dispensers ($P < 0.05$ ground, $P > 0.05$ canopy, Figs. 7.20 and 7.21). P values from the 1996 field season for carabids and aphids are shown in Appendix 3, Tables 10.7-10.

Throughout the field season so few coccinellids and Syrphids were trapped in the plots that statistical analysis was impossible (Appendix 4).

7.3.2 1997

There was no significant difference between the numbers of carabids trapped in each of the 3 fields ($P > 0.05$, Fig. 7.22). There was a significant difference in the mean number of carabids trapped at the three distances ($P < 0.05$, Fig. 7.23), with fewer number of active carabids trapped at 0-10m than either 45-55m or 90-100m. Slightly more carabids were trapped at 45-55m than 90-100m but this increase was not significant. There was a significant difference in the numbers of aphids trapped in the three fields with significantly more aphids in field 2 relative to fields 1 and 3

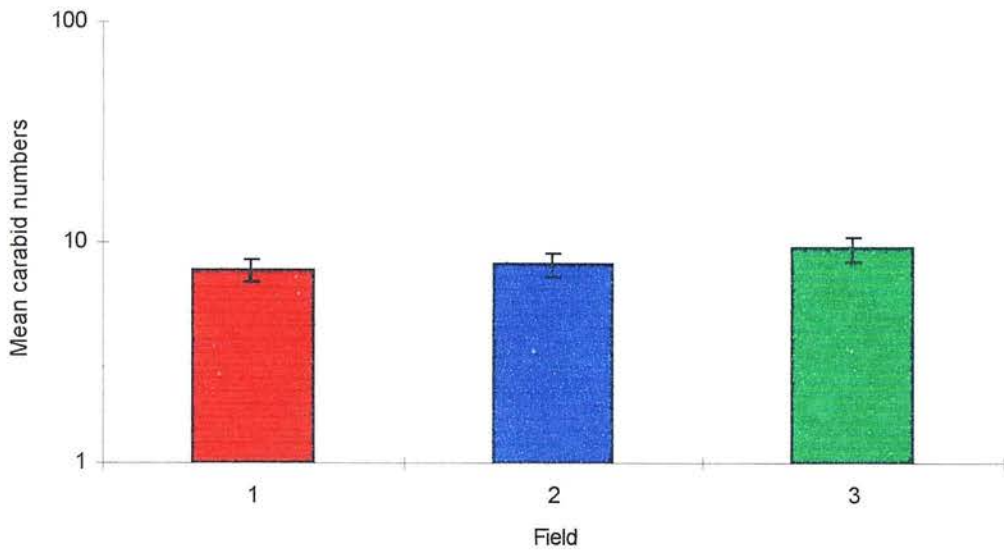


Fig. 7.22 Mean numbers of carabids (\pm SE) trapped per week in each field in 1997 from randomised linear model (sample size=17)

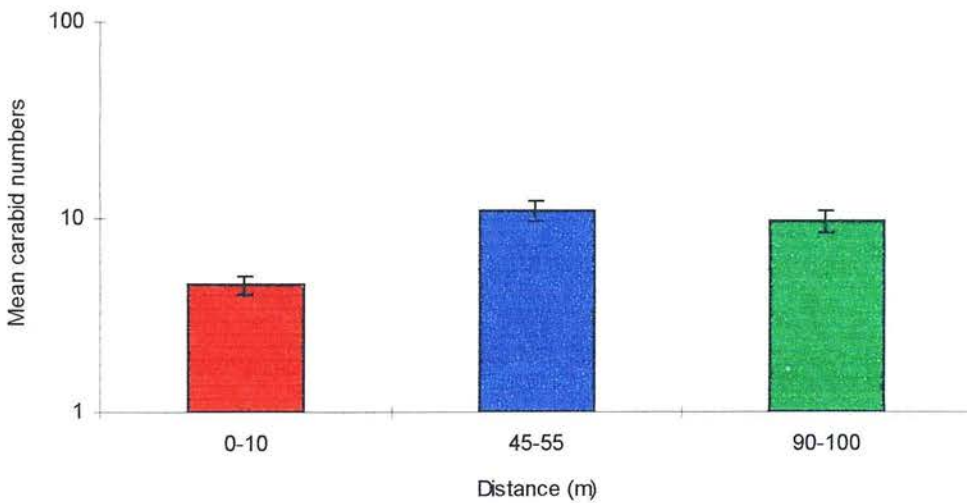


Fig. 7.23 Mean numbers of carabids (\pm SE) trapped per week in at each distance in 1997 from randomised linear model (sample size=17).

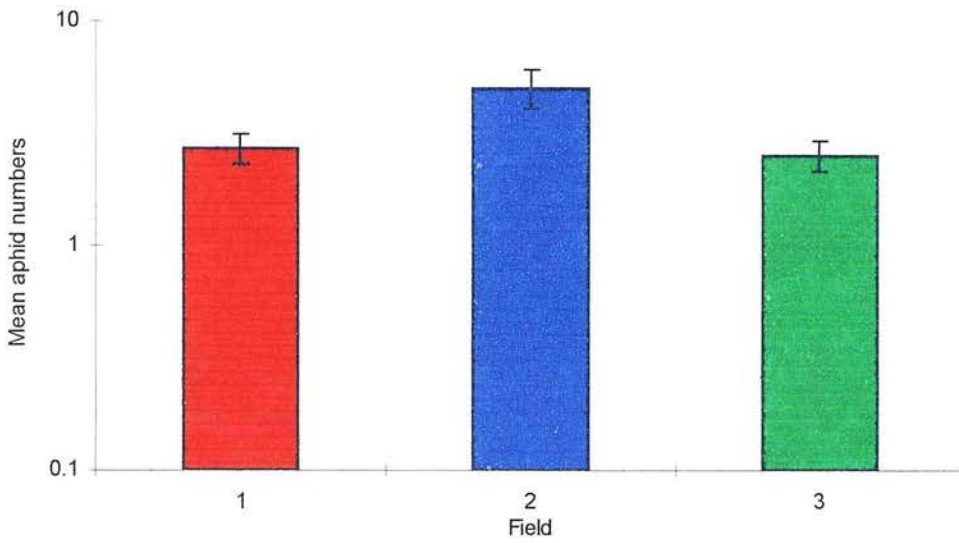


Fig. 7.24 Mean numbers of aphids (\pm SE) trapped per week in each field in 1997 from randomised linear model (sample size=17)

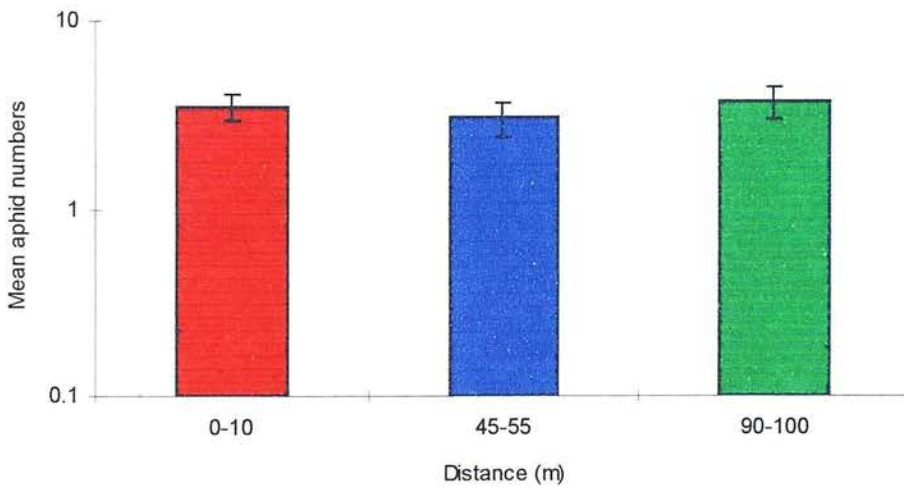


Fig. 7.25 Mean numbers of aphids (\pm SE) trapped per week in at each distance in 1997 from randomised linear model (sample size=17).

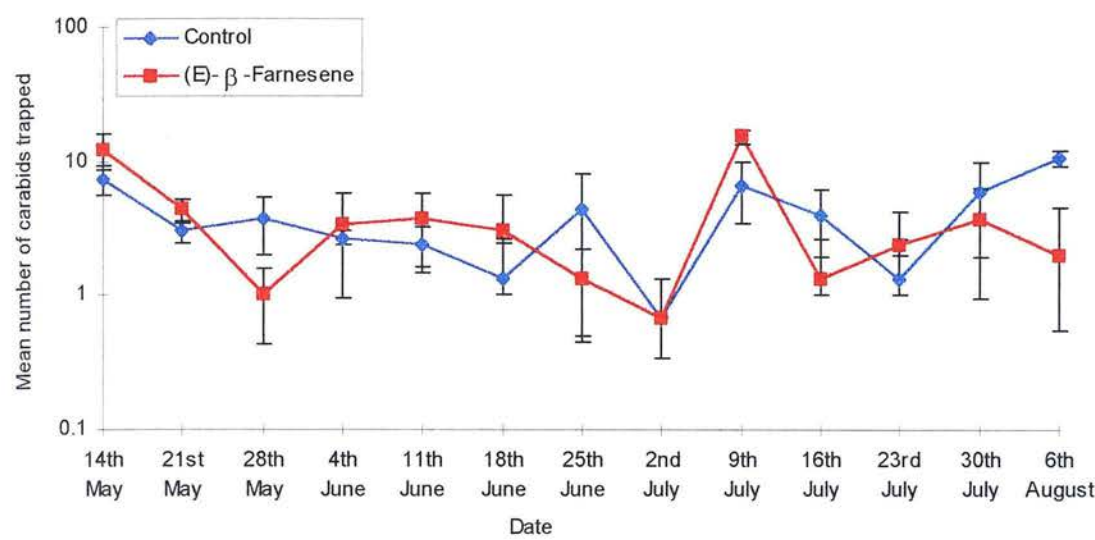


Fig. 7.26 Mean number of carabids (\pm SE) trapped at 0-10m in 1997 with (E)-β-farnesene treatment (sample size=3).

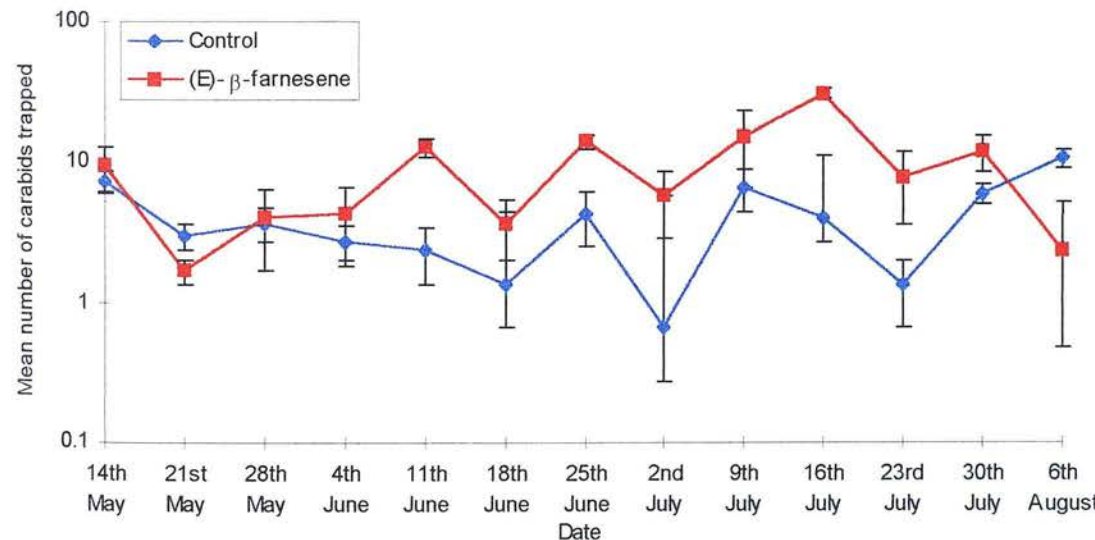


Fig. 7.27 Mean number of carabids (\pm SE) trapped at 45-55m in 1997 with (E)-β-farnesene treatment (sample size=3).

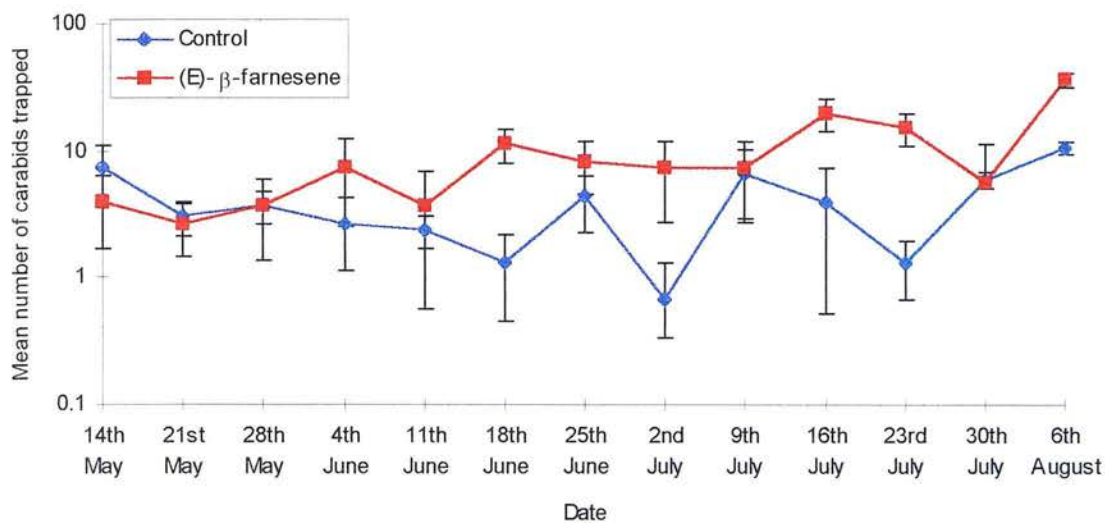


Fig. 7.28 Mean number of carabids (\pm SE) trapped at 90-100m in 1997 with (E)- β -farnesene treatment (sample size=3).

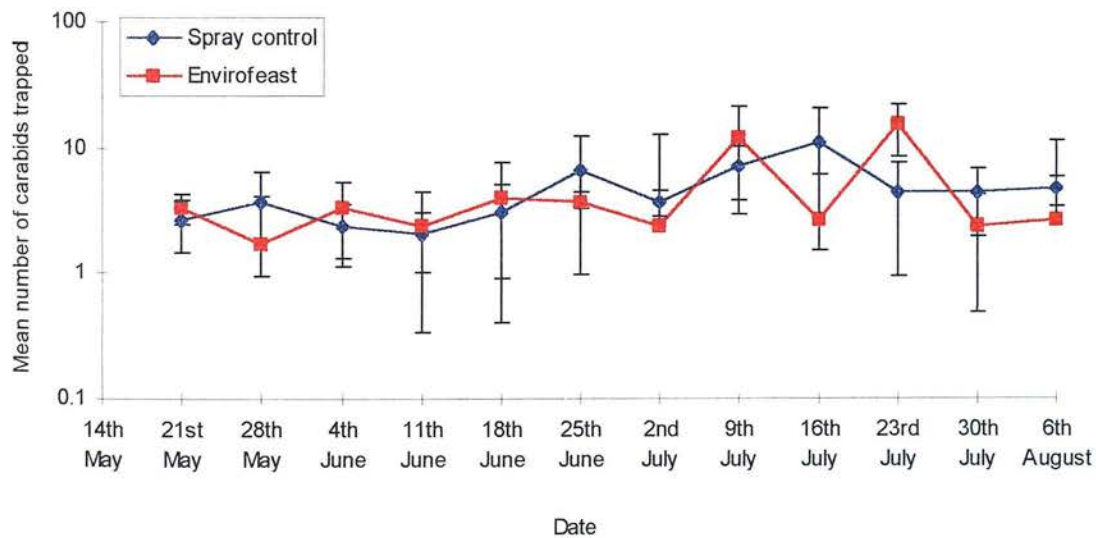


Fig. 7.29 Mean number of carabids (\pm SE) trapped at 0-10m in 1997 with EnvirofeastTM treatment (sample size=3).

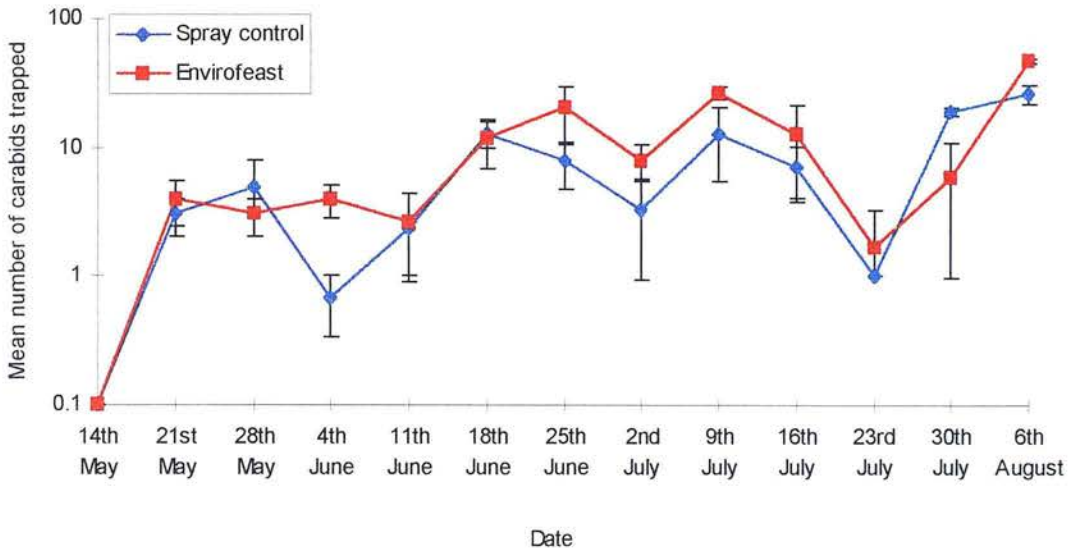


Fig. 7.30 Mean number of carabids (\pm SE) trapped at 45-55m in 1997 with EnvirofeastTM treatment (sample size=3).

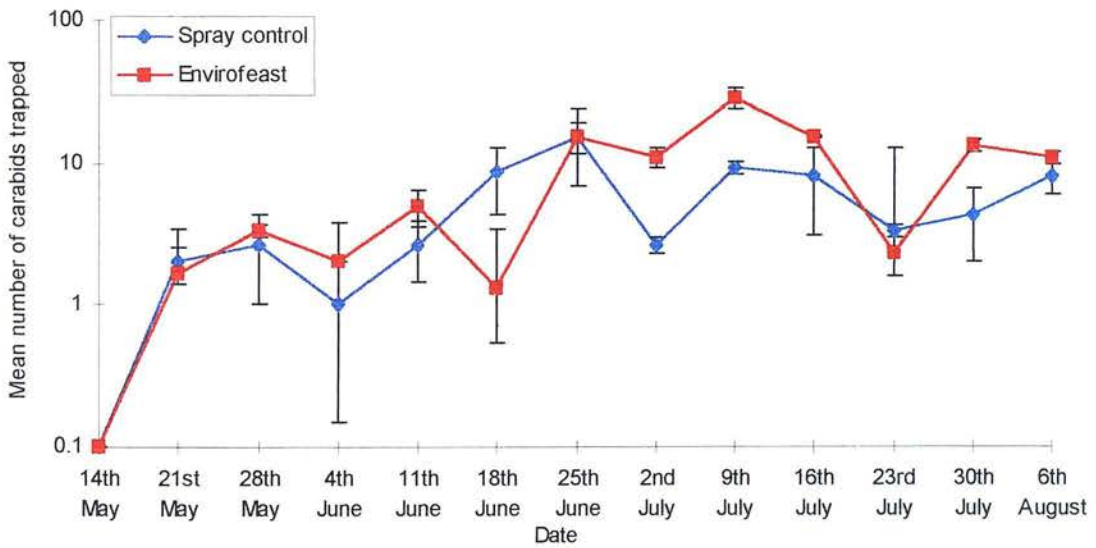


Fig. 7.31 Mean number of carabids (\pm SE) trapped at 90-100m in 1997 with EnvirofeastTM treatment (sample size=3).

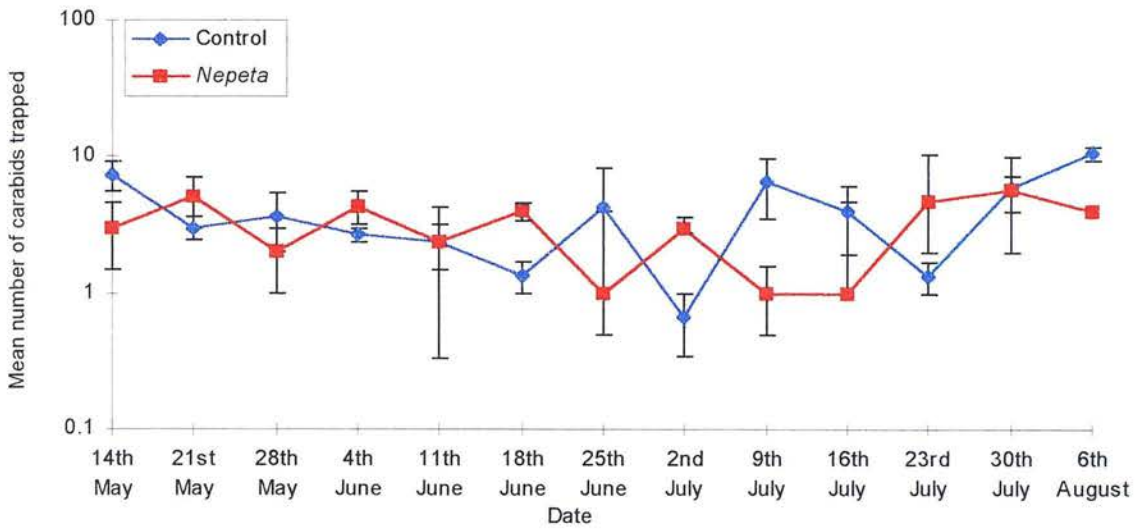


Fig. 7.32 Mean number of carabids (\pm SE) trapped at 0-10m in 1997 with *Nepeta* extract treatment (sample size=3).

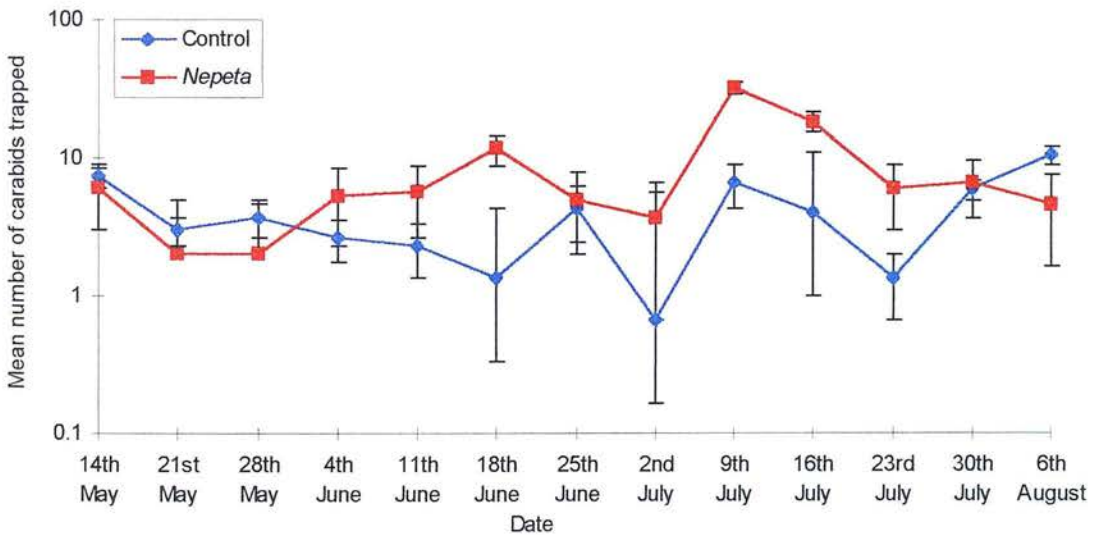


Fig. 7.33 Mean number of carabids (\pm SE) trapped at 45-55m in 1997 with *Nepeta* extract treatment (sample size=3).

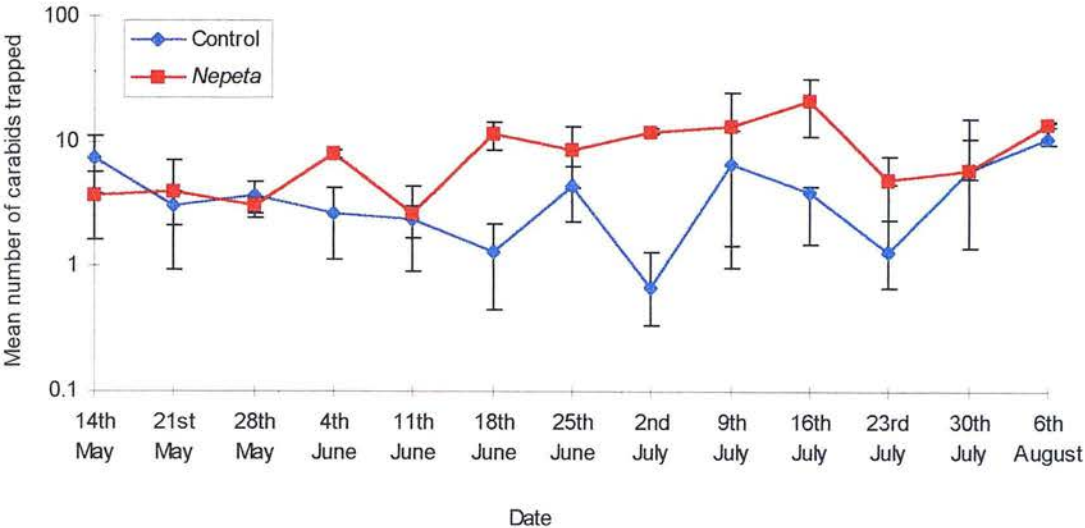


Fig. 7.34 Mean number of carabids (\pm SE) trapped at 90-100m in 1997 with *Nepeta* extract treatment (sample size=3).

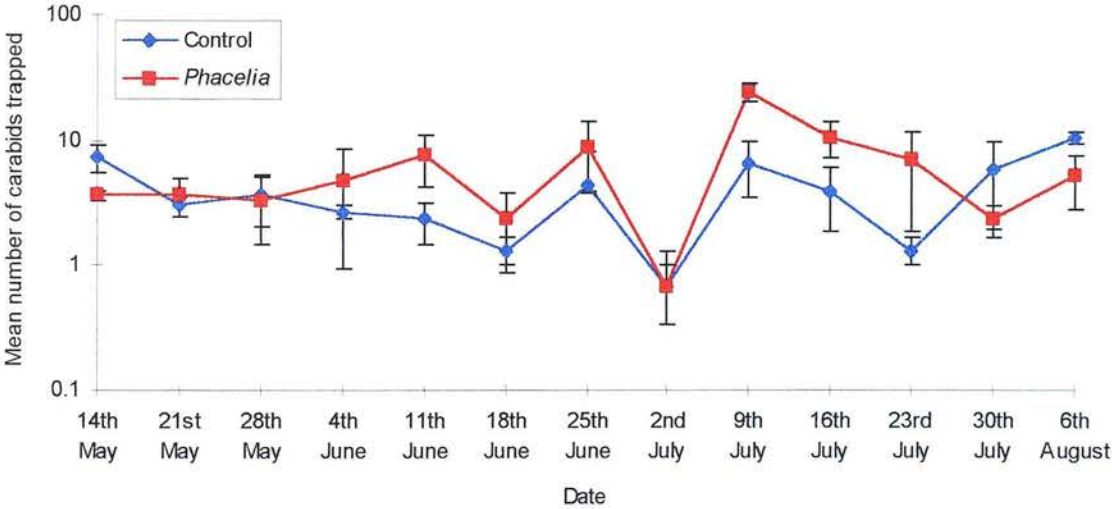


Fig. 7.35 Mean number of carabids (\pm SE) trapped at 0-10m in 1997 with *Phacelia* flower extract treatment (sample size=3).

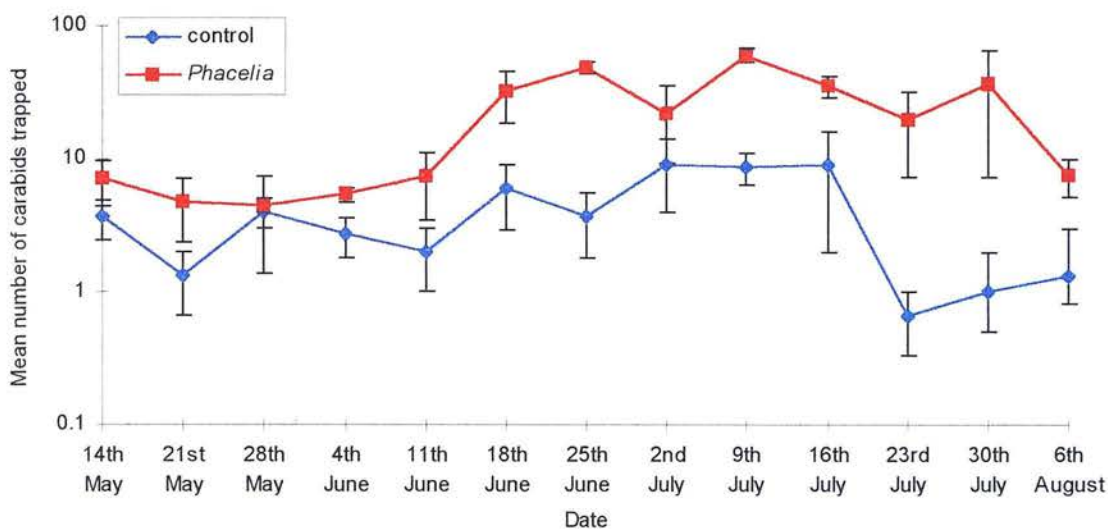


Fig. 7.36 Mean number of carabids (\pm SE) trapped at 45-55m in 1997 with *Phacelia* flower extract treatment (sample size=3).

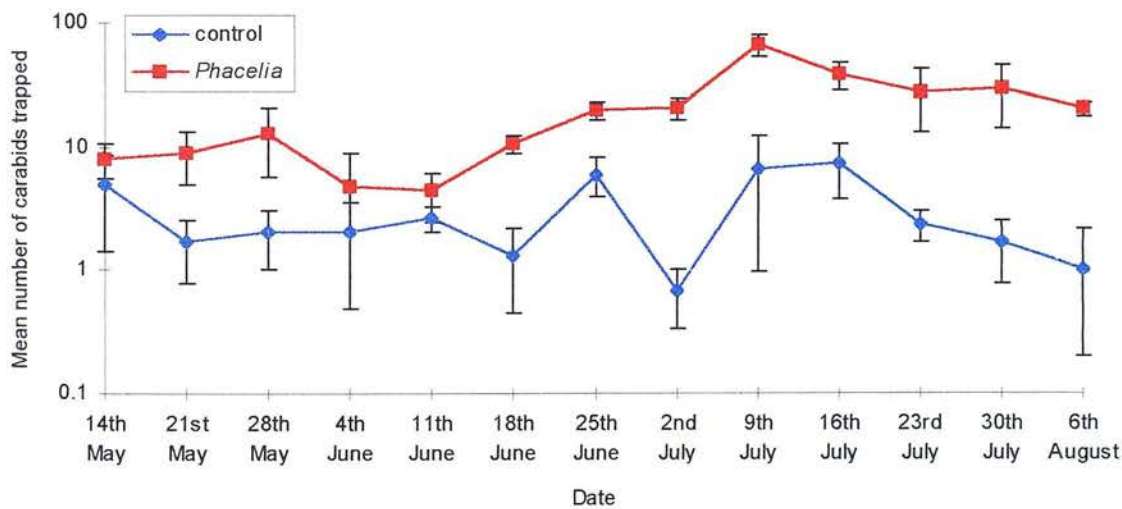


Fig. 7.37 Mean number of carabids (\pm SE) trapped at 90-100m in 1997 with *Phacelia* flower extract treatment (sample size=3).

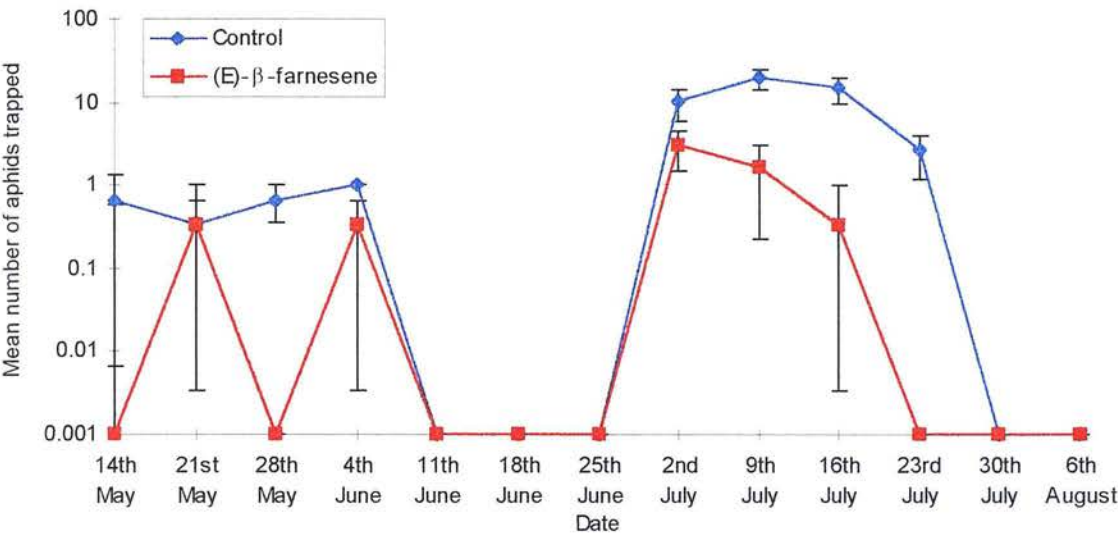


Fig. 7.38 Mean number of aphids (± SE) trapped at 0-10m in 1997 with (E)-β-farnesene treatment (sample size=3).

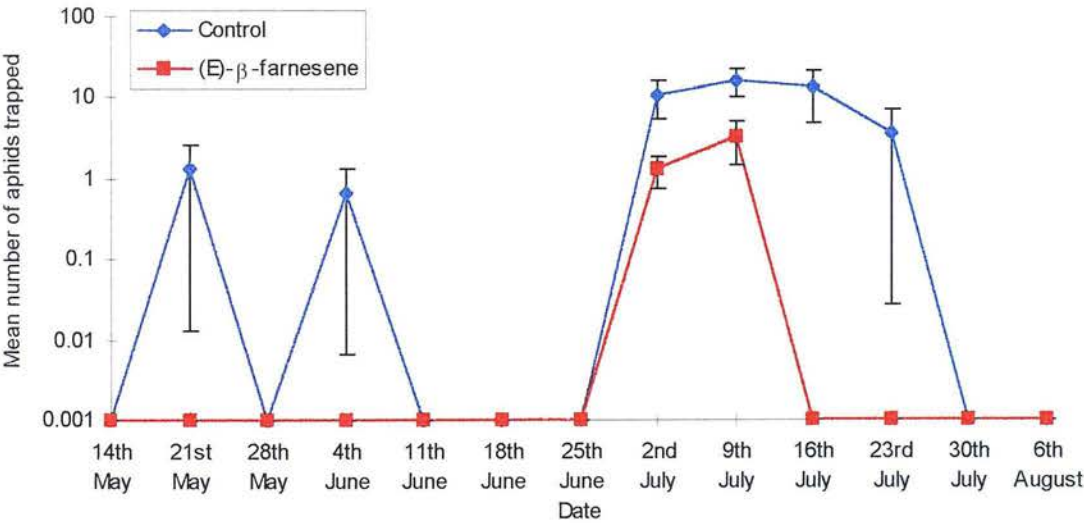


Fig. 7.39 Mean number of aphids (± SE) trapped at 45-55m in 1997 with (E)-β-farnesene treatment (sample size=3).

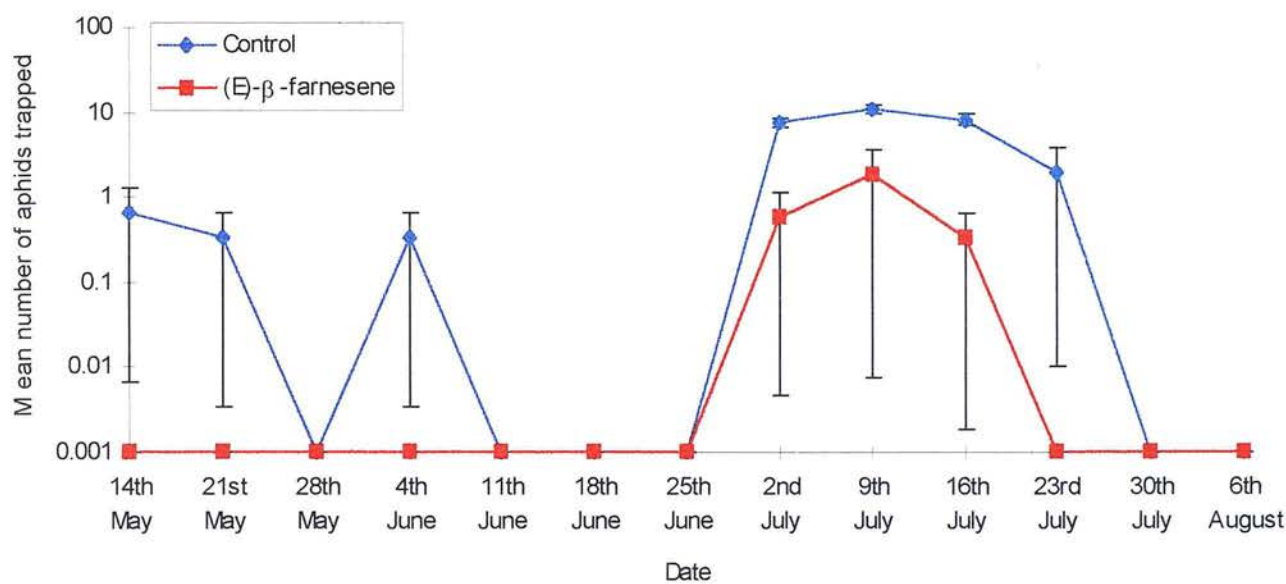


Fig. 7.40 Mean number of aphids (\pm SE) trapped at 90-100m in 1997 with (E)-β-farnesene treatment (sample size=3).

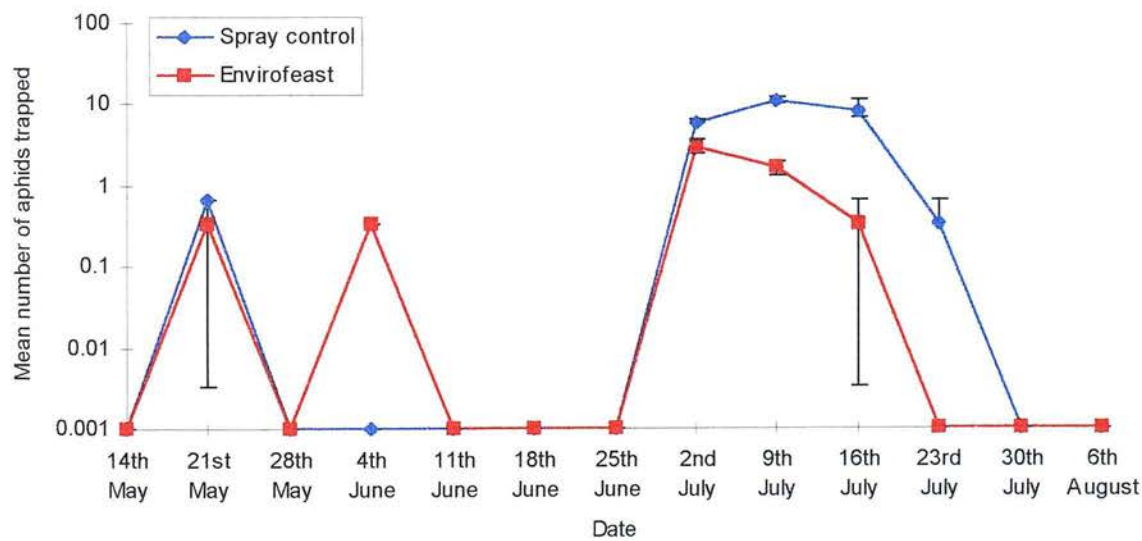


Fig. 7.41 Mean number of aphids (\pm SE) trapped at 0-10m in 1997 with Envirofeast™ treatment (sample size=3).

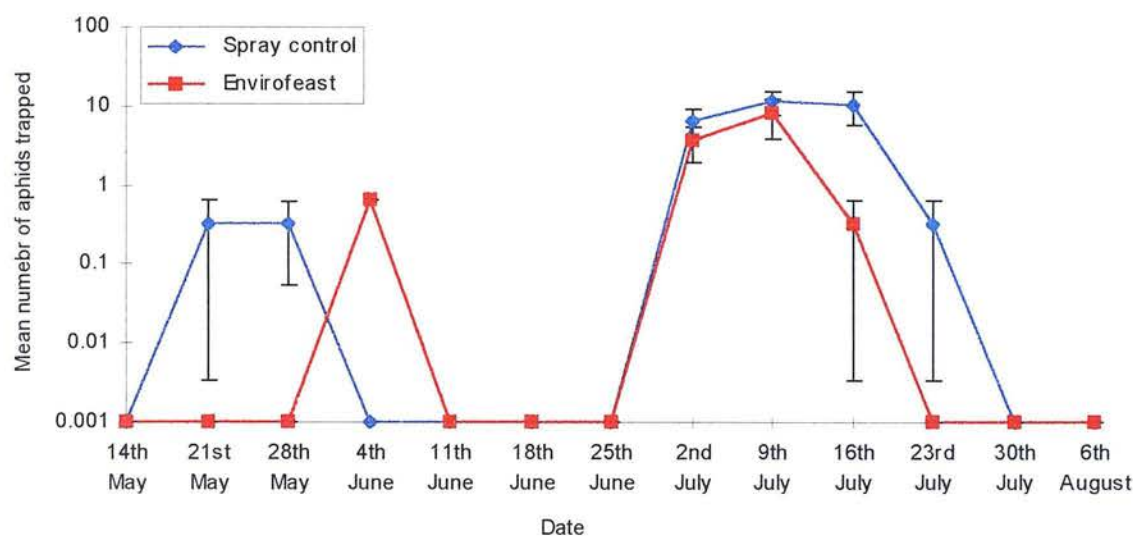


Fig. 7.42 Mean number of aphids (\pm SE) trapped at 45-55m in 1997 with Envirofeast™ treatment (sample size=3).

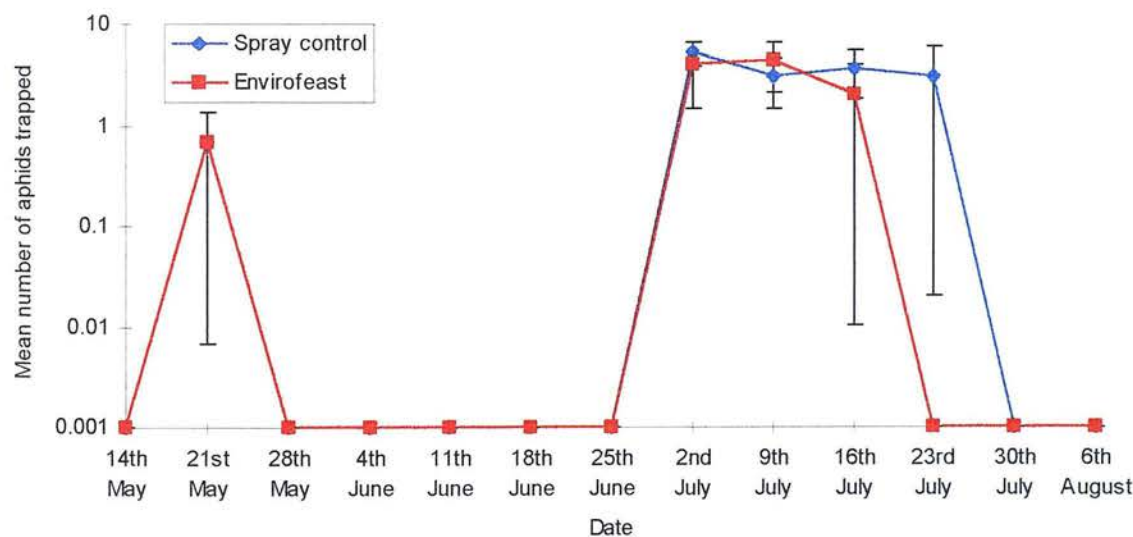


Fig. 7.43 Mean number of aphids (\pm SE) trapped at 90-100m in 1997 with Envirofeast™ treatment (sample size=3).

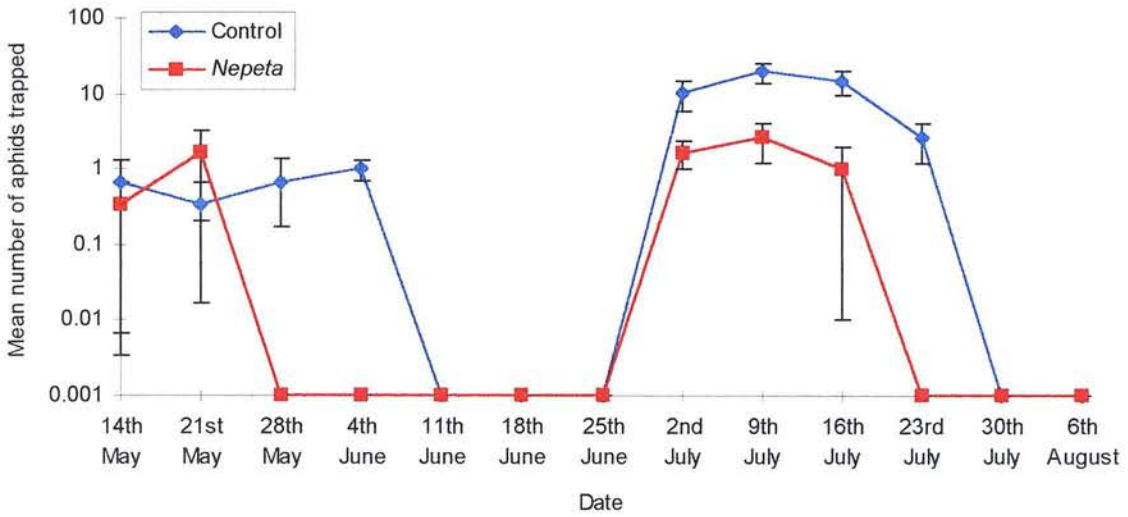


Fig. 7.44 Mean number of aphids (\pm SE) trapped at 0-10m in 1997 with *Nepeta* extract treatment (sample size=3).

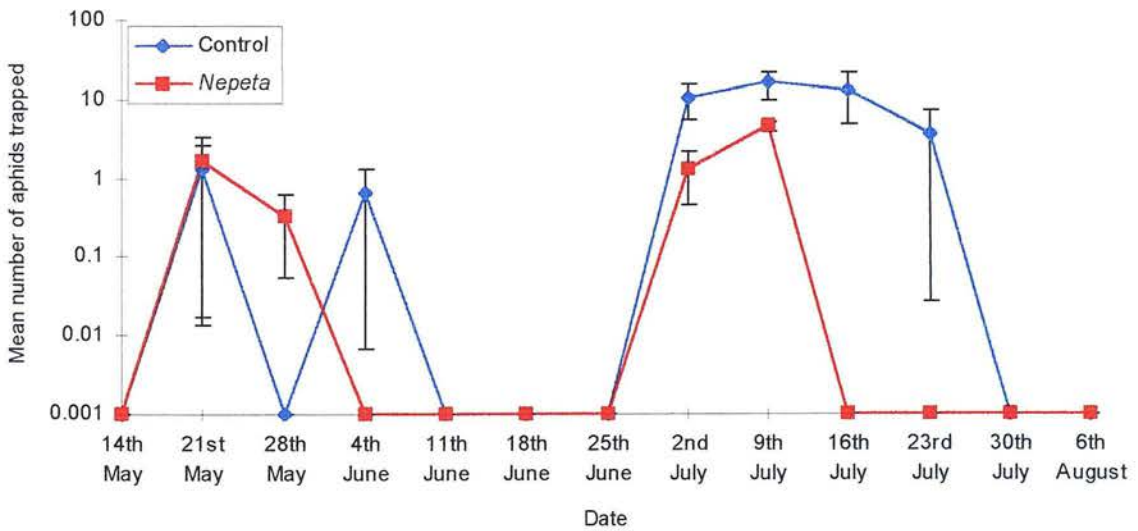


Fig. 7.45 Mean number of aphids (\pm SE) trapped at 45-55m in 1997 with *Nepeta* extract treatment (sample size=3).

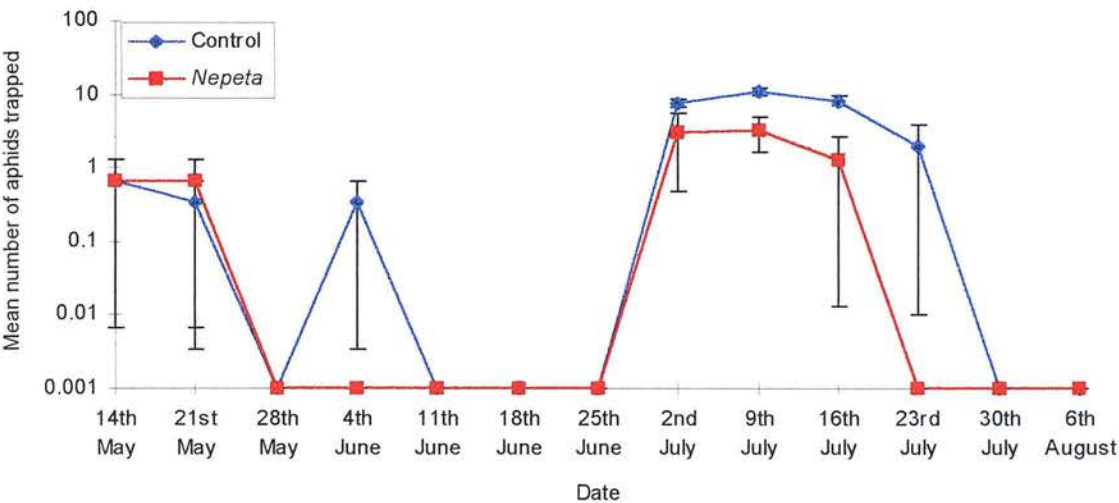


Fig. 7.46 Mean number of aphids (\pm SE) trapped at 90-100m in 1997 with *Nepeta* extract treatment (sample size=3).

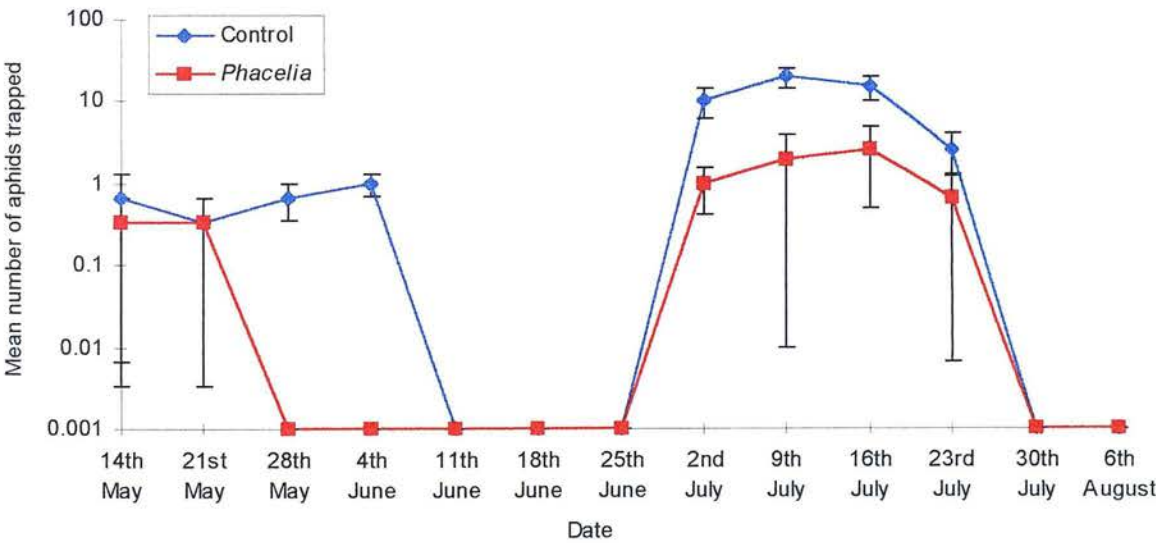


Fig. 7.47 Mean number of aphids (\pm SE) trapped at 0-10m in 1997 with *Phacelia* flower extract treatment (sample size=3).

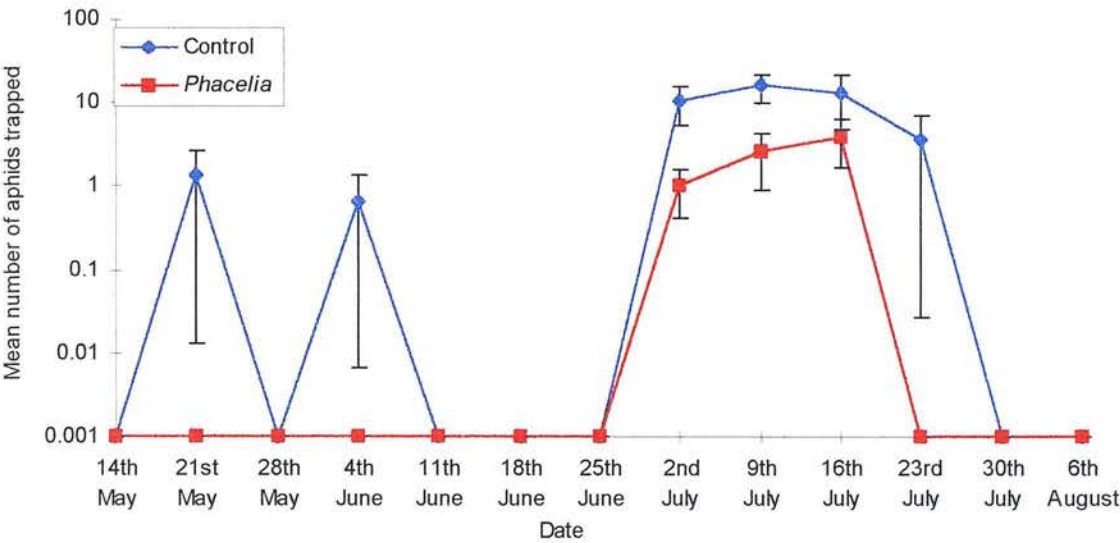


Fig. 7.48 Mean number of aphids (\pm SE) trapped at 45-55m in 1997 with *Phacelia* flower extract treatment (sample size=3).

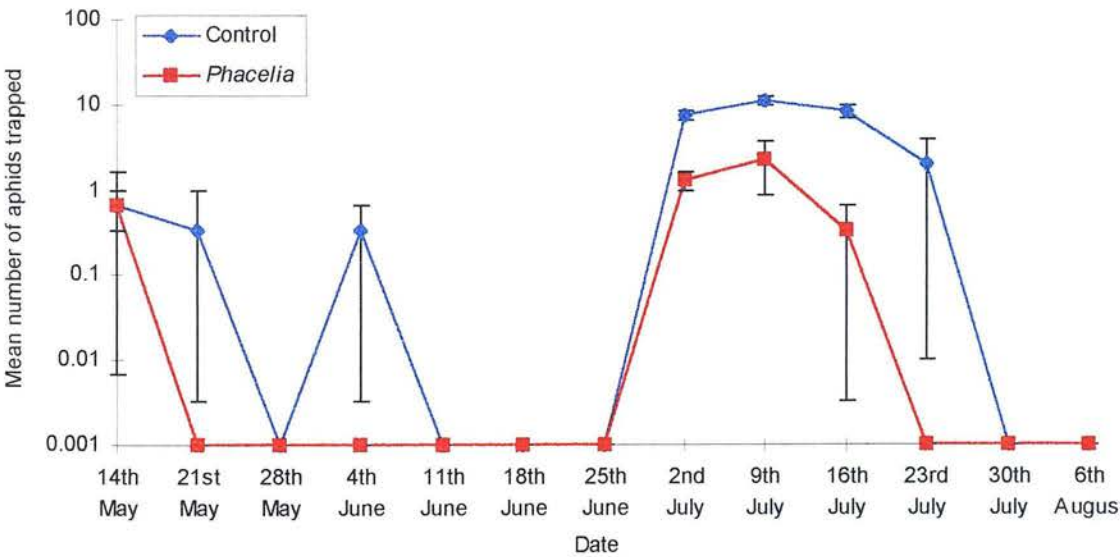


Fig. 7.49 Mean number of aphids (\pm SE) trapped at 90-100m in 1997 with *Phacelia* flower extract treatment (sample size=3).

($P < 0.05$, Fig. 7.24). However, there was no significant difference between the mean numbers of aphids trapped at any of the three distances ($P > 0.05$, Fig. 7.25).

With the (E)- β -farnesene, *Nepeta* extract and *Phacelia* flower extract treatments, there was no significant increase in carabid numbers between 0-10m into the crop relative to the control ($P > 0.05$, Figs 7.26, 7.32 and 7.35). However, with all three treatments there was an increase in the number of active carabids at 45-55m ($P < 0.01$) and at 90-100m into the crop ($P < 0.01$, $P < 0.01$, and $P < 0.001$ respectively, Figs. 7.27-28 and 7.32-37) relative to the control.

With Envirofeast™ there was no significant increase in carabid numbers at 0-10m, 45-55m, or 90-100m relative to the spray control plots ($P > 0.05$ Figs. 7.29-31).

With (E)- β -farnesene, *Nepeta* extract and *Phacelia* flower extract there was a significant decrease in aphid numbers at 0-10m ($P < 0.001$, $P < 0.01$ and $P < 0.001$ respectively, Figs. 7.38, 7.44 and 7.47), 45-55m ($P < 0.01$, Figs. 7.39, 7.45, and 7.48), and 90-100m ($P < 0.001$, $P < 0.01$, $P < 0.001$ respectively, Figs. 7.40, 7.46 and 7.49). With the Envirofeast™ treatment there was only a significant fall in aphid numbers at 0-10m ($P < 0.001$, Fig. 7.41), with no significant difference in aphid numbers at 45-55m and 90-100m relative to the controls ($P = 0.055$, and $P > 0.05$, Figs. 7.42-43). P values from the 1997 field season for carabids and aphids are shown in Appendix 5, Tables 10.11-14.

7.4 Discussion

7.4.1 1996

In 1996 the variation in the weekly beetle trap catches follows the patterns of carabid population growth as shown by Luff (1987): the first peak is due to the emergence of carabids from overwintering and the high activity of spring breeding species, the population then declines as the adults of the spring breeders begin to die and some species of the autumn breeders enter a developmental diapause such as found in *N. brevicollis*. The activity of the beetles then begins to increase again at the beginning of August, as the activity of the autumn breeders increases at the end of summer and into their main reproductive period.

Both artificial honeydew and a component of natural honeydews (L-tryptophan) were found not to have a significant effect on the number of carabids in both winter wheat and spring barley fields. McEwen *et al* (1994) found that L- tryptophan caused an increase in the numbers of lacewings (*C. carnea*) and a subsequent decline in the number of pest species in olive groves when sprayed onto the trees. Ben Saad & Bishop (1976) also found that artificial honeydew could cause a significant increase in the number of predators when applied to potato crops. However, in both studies the predators monitored differed from the carabids in this study, and there is no evidence that artificial honeydews and L-tryptophan spray act as cues for foraging carabids searching for resources.

Phacelia extracts did lead to a significant increase in the number of carabids in the fields. This supports the finding of the electrophysiological and behavioural assays (Chapters 4, 5 and 6) and also with Cowgill (1993b) and Hickman & Wratten (1996),

who found that strips of *Phacelia* planted around the edges of fields increased the amount of hoverflies and their larvae within the crop. *Phacelia* extract was most attractive to carabids when placed in ground level dispensers. Carabids are mainly ground dwelling species, and therefore if dispensers release volatiles closer to the ground there is an increased chance that they will be detected by carabids.

Nepeta extract also led to an increase in the number of beetles present in the plots. Although the beetles did demonstrate an electrophysiological response to *Nepeta* extract, in behavioural trials in the laboratory carabids exhibited a slight, but not significant, behavioural response to *Nepeta* extract (Chapter 6). This slight response may be enough to cause a significant increase in the number of beetles over time under field conditions.

(E)- β -farnesene led to an increase in the number of polyphagous carabids in both winter wheat and spring barley. However, the greatest effect was produced with (E)- β -farnesene in canopy level dispensers, with ground level dispensers producing a relatively smaller (not significant) increase in beetle numbers. This may indicate that *Phacelia* and *Nepeta* are odour cues to help the beetle locate the habitat where resources may be located. As the carabids are mainly ground hunters, during habitat location they will follow chemical cues diffusing just along ground level. The use of habitat location cues has been previously demonstrated in carabids (Evans, 1988).

With artificial honeydew and L-tryptophan in winter wheat and Envirofeast™ in spring barley, there was a significant decline in aphid numbers relative to the control plots which supports the study of McEwen (1994), even though there was not a significant increase in the numbers of carabids in the plots. Therefore, the effects of

artificial honeydew, L-tryptophan and Envirofeast™ on aphid populations may either be due to other non-carabid predators, which is not supported by the present study, or the compounds may have a direct effect on the behaviour of the aphids themselves, causing the reduction in aphid numbers seen by McEwen (1994).

There was a decline in the number of aphids with both ground and canopy level dispensers of (E)- β -farnesene, *Nepeta* and *Phacelia* flower extracts. However, for both *Nepeta* and *Phacelia* extract the greatest decline in aphid numbers occurred with ground level dispensers with a relatively smaller decline with the canopy level dispensers. This follows the pattern of carabid activity, with greatest activity occurring with the ground level dispensers. This suggests that the beetles may be responsible for the decline in aphid numbers. However, with (E)- β -farnesene the decline in aphid numbers was the same with both ground and canopy level dispensers, although there was only a significant increase in beetle numbers with the canopy level dispensers. This may be because (E)- β -farnesene is the major component of the aphid alarm pheromone which when detected by the aphids induces them to initiate an alarm response, by either stopping feeding and walking away from the plant, or to fall off the plant completely (Dawson *et al.*, 1987). The aphids which fall off the plant are then more vulnerable to predation by ground dwelling predators. So (E)- β -farnesene may reduce aphid numbers in two ways, by attracting in predators and by directly affecting aphid behaviour. However, in greenhouse trials pea aphids, *Acyrtosiphon pisum* Harris, became habituated to (E)- β -farnesene after approximately 30 minutes of exposure to the semiochemical

(Elagamy and Haynes, 1992). Therefore, habituation may reduce the effects of (E)- β -farnesene on aphid behaviour, making it unsuitable for use in IPM systems which rely on behavioural disruption to control aphid populations, but it may still be used to attract predators to the field.

7.4.2 1997

In 1997 there were significant differences in the number of beetles trapped at the three distances from the edge of the crop. However, this differed from the findings of Kielty *et al.*, (1995), who found that the numbers of carabids throughout the field system occurred in a gradient from the field boundary to the centre of the field, with the highest numbers occurring around the edge of the field. In the current study, greatest numbers of carabids occurred 45-55m into the field. This may have been due to a variety of factors. It may have been that colonisation of the fields in Kielty's (1995) studies by carabids occurred from the field edges. However, in this study there may have been less migration from outside the field and colonisation took place from overwintering beetles within the field boundaries.

The increased numbers of carabids located in the centre of the field may also be due to an effect of the treatment. Foraging predators move up a concentration gradient toward areas of increasing concentration of the semiochemical (Dawson *et al.*, 1993), they may also carry out positive anemotaxis in an effort to locate the source of the stimulus (Vet *et al.*, 1983). On reaching the dispenser the beetle may then contact the odour plume from the next dispenser, and move deeper into the field, following the lines of dispensers.

(E)- β -farnesene, *Nepeta* extract and *Phacelia* extract all lead to an increase in the number of carabids caught in the fields at 45-55m and 90-100m from the edge of the crop, however, there was no significant increase in carabid numbers at 0-10m into the field with all three treatments. This may be because beetles in this area may have moved deeper into the crop following the line of semiochemical dispensers, or returned to the field boundary for shelter.

(E)- β -farnesene has been identified as a major component of the alarm pheromone of several aphid species (Herrbach, 1992), and has been demonstrated to be attractive to carabids in laboratory studies (Kielty *et al.*, 1995), and to other insects in the field (Lilley *et al.*, 1994b). Similarly, the green leaf volatiles of *Nepeta cataria*, nepetalactol and nepetalactone, have been identified as the active components of the sex pheromone of several economically important aphid species (Tumlinson *et al.*, 1991; Hardie *et al.*, 1994). So both (E)- β -farnesene and *Nepeta* represent direct location cues that enable the carabids to find prey.

Phacelia provides few resources for most carabid species, as they obtain resources from the prey. Thus *Phacelia* may act as a habitat location cue, enabling the beetle to find habitat where prey species might be present (Kirkland, Evans and Lola, 1998).

Although there was an increase in carabid numbers in the plots with Envirofeast™ this was not significant. However, Envirofeast™ was developed in Australia, for use on a variety of pest species on cotton crops (Mensah, 1997b). Because Australia has a relatively low rainfall throughout the year Envirofeast™ was not formulated to be water resistant (Mensah, 1997b). There was a high rainfall on the crops during the

summer of 1997 which may have washed away the Envirofeast™ reducing its effectiveness.

With (E)- β -farnesene, *Nepeta* extract and *Phacelia* flower extract there was a significant decline in aphid numbers at all distances into the crop, even though significant increases in carabid numbers were only detected at 45-55m and 90-100m. This could possibly be due to two effects: (E)- β -farnesene and nepetalactol and nepetalactone found in *Nepeta* extract are components of aphid pheromone systems and so may have a direct effect on the behaviour of the aphids themselves (Dawson *et al.*, 1990). However, the previous years results suggested that *Phacelia* does not have a behavioural effect on aphids themselves, although there is still a decline in aphid numbers at 0-10m with the *Phacelia* treatment even though there is no significant increase in carabid numbers at this distance. This may be because an increased number of carabids pass through this area quickly following the odour fields from the dispensers, and as they pass through this area they feed on the aphids reducing aphid numbers in the area, even though the carabids are not detected in the area. Further experiments need to be carried out on this relationship to determine the mechanism of reduction of aphid numbers.

It may be possible to introduce a semiochemical component into an integrated pest management system. The semiochemical would attract polyphagous predators, increasing their number within an arable field which would then have a greater impact on pest populations. This may lead to a reduction in the need for applications of chemical insecticides to achieve effective control. Chemical insecticides could be used early in the season to control pest numbers before populations of predators

become established. The semiochemicals introduced into the field could attract in predators which could then control the reduced pest populations throughout the rest of the season, alleviating the need for a second pesticide application.

7.5 Summary

- In 1996 (E)- β -farnesene, *Nepeta* extract and *Phacelia* flower extract all lead to a significant increase in the number of carabids in treated plots relative to control plots
- However with *Nepeta* extract and *Phacalia* flower extract greatest increase in beetle numbers occurred with ground level dispensers, but with (E)- β -farnesene greatest increase in beetle numbers occurred with canopy level dispensers.
- In 1996 in the treated plots for each increase in carabid numbers there was a corresponding fall in aphid numbers relative to the controls.
- In 1997 (E)- β -farnesene, *Nepeta extract* and *Phacelia* flower extract all lead to a significant increase in beetle numbers at 45-55m and 90-100m from the field edge but not at 0-10m from the field edge, relative to the control plots.
- Aphid numbers declined at all distances from the field edge in treated plots relative to the controls.

Chapter 8

Mechanism of reduction of pest numbers

Chapter 8. Mechanisms of reduction of pest numbers

8.1 Introduction

Predation by arthropods is very difficult to monitor in the field because the insects are small and difficult to see in the vegetation and because predation often takes place at night (Sopp *et al.*, 1992). Therefore estimating the role of predators in the regulation of pest populations and the structuring of communities is complicated (Sopp *et al.*, 1992).

Early methods for analysing predation relied on key factor analysis (Varley & Gradwell 1960), where pest and predator populations were measured in the field, by a combination of direct observation and trapping. Other factors involved in pest mortality, such as consumption rates, availability of prey species to predators and background mortality of prey (Finch & Elliot, 1992), were analysed in the laboratory. These factors were used in a model for assessing the role each factor plays in mortality, which can be used to give an estimate of predation in the field. This approach frequently identifies predators as major sources of mortality in insect populations (Stiling, 1988), but is unable to quantify the mortality or identify the predators (Sopp *et al.*, 1992). Using this system predation rates are estimated under optimal conditions measured in the laboratory, and not often present under field conditions, and mortality not accounted for by other factors is often assumed to result from predation, with little evidence to support this (Sanchez & Liljestrom, 1986). This may lead to an overestimation of rates of predation in the field. One way to overcome this is to link populations studies from the field with a biochemical

assay which can detect and quantify the remains of prey species in the gut contents of captured predators (Symondson & Liddel, 1993; Sunderland, 1994).

Semiochemicals attractive to arthropod predators in both laboratory and field trials have previously been identified and assessed (Chapters 5-7). When the semiochemicals were situated in the field two main effects were observed; an increase in predator activity and a decrease in the number of aphids present in the plots containing the semiochemical. However, the reduction in aphid numbers could occur by two different mechanisms. The semiochemicals could directly effect the behaviour of the pests themselves, as in the case of (E)- β -farnesene, a component of the aphid alarm pheromone, which induces a general alarm response in aphids (Dawson *et al.*, 1987). Or the semiochemicals may attract predators which use the chemicals as prey or habitat location cues, thereby reducing the number of pest species by predation. In previous, studies *Phacelia* flower extract has been identified as the most effective semiochemical to attract predators in the field (Chapter 7).

The mechanism of reduction in pest numbers using *Phacelia* extract was investigated using exclusion experiments in the field linked with ELISAs of beetle gut contents.

Exclusion experiments using plastic barriers have been used successfully in previous studies to look at levels of predation in the field. In a study by Holland & Thomas (1997), barriers were employed to restrict entry of carabids into field plots. Although the barriers did not completely exclude carabids from the plot they did reduce their numbers. In the barriered plots there was a corresponding rise in aphid populations (Holland & Thomas, 1997).

8.1.1 Assessing predation

Early biochemical methods for estimating predation on agricultural pests focused on the use of precipitin tests, where chemicals were used to precipitate out prey species proteins from a solution of the predator gut contents (Frank, 1979). This test simply used the number of positive results (i.e. those in which a precipitate is seen) as an indicator of predation. However, this test could be biased by differential variation in the rates of antigen deactivation in different predators (Sunderland, 1987), and could not be used to quantify the amount of prey remains in individual predator guts (Sunderland, 1994). This therefore provided estimation of consumption rates useful in energetic studies, but did not provide an estimation of predation rates needed for assessments of pest control by predators (Sunderland, 1994).

This method was subsequently improved with the development and use of ELISAs (enzyme linked immunosorbant assays), which could be used to not only provide information on the proportion of a population of predators which are preying on a pest species, but also on the quantity of prey biomass in an individual predator gut (Sunderland, 1994). At first polyclonal antibodies were used in the assays, but development and improvement of monoclonal antibodies has increased the specificity of the assay enabling more accurate information about predation on agricultural pest species to be obtained (Symondson & Liddell, 1996).

Pest proteins from the gut contents of the predator anneal to the sides of a microtitre well (Fig. 8.1). Antibodies raised in mice and specific to the antigen are then used to bind to the antigen. A rabbit antimouse antibody conjugated with a peroxidase enzyme then bind to the mouse antibodies.

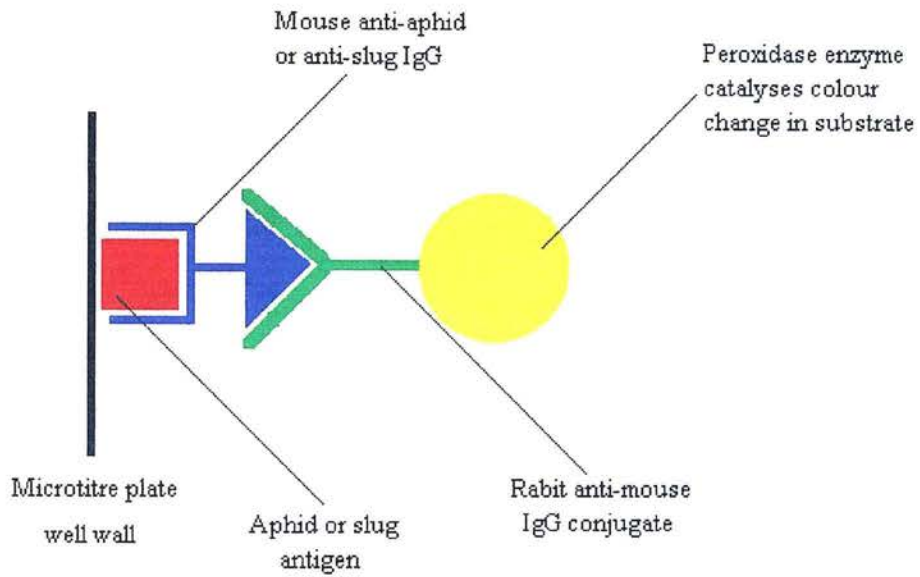


Fig. 8.1 Diagrammatic representation of the Enzyme Linked Immunosorbant Assay

When the peroxidase enzyme is provided with a substrate it produces a colour change in the well, the intensity of which can be used to quantify the amount of enzyme and therefore the amount of antigen in the sample, thus enabling rates of predation to be estimated (Symondson & Liddell, 1996, Fig. 8.1). This technique is now the most established method used in predation studies (Stuart & Greenstone 1990), and has been used to provide estimates of predation in several systems including control of the lepidopteran pest *Heliocoverpa zea* Boddie (Greenstone & Morgan, 1989), the heteropteran *Lygus hesperus* Knight (Hagler *et al.*, 1991), and the mollusc *Deroceras reticulatum* Müller (Symondson & Liddell, 1993).

8.2 Materials and methods

8.2.1 Field trials

Field trials were set up according to the modified method of Holland & Thomas (1997), and the method for field trials in 1996 (Chapter 7). Three winter wheat fields (cv. Riband) were selected for the trials located at Boghall Farm (Midlothian, UK, OS map 66, 653242). The fields were selected because they were approximately the same size (10.28, 10.57, and 11.03 hectares respectively). Four 10m x 10m plots were marked off in each field using canes (Fig. 8.2). The plots consisted of dispensers releasing methanol (control), *Phacelia* extract, methanol (barriered) and *Phacelia* extract (barriered).

For the two barriered plots, strips of 1.5mm thick polythene (11m x 60cm) were sunk 30cm into the ground leaving 30cm protruding above the surface, around the perimeter of the plot. This prevented carabids in the ground from tunnelling under or climbing over the polythene and entering the plots. At each corner, the polythene overlapped 0.5m and the ends were tied together to seal any gaps. The polythene was supported by canes placed every 0.5m, which prevented it blowing flat in the wind. The vegetation was removed for 10cm around the perimeter of the plots to prevent carabids climbing up the plants and into the plots.

Two weeks before the trials, and before the carabids emerged from overwintering, the 2 plots with plastic barriers were sprayed with chloropyrifos (Dursban 4, Dow Elanco), a persistent soil insecticide, to kill any carabids overwintering in the plots.

Five dry covered pitfall traps were placed at random in each of the plots, to measure the activity density of carabid populations, and also to trap out any carabids remaining in the barriered plots after the insecticide treatments. A wicked semiochemical dispenser was placed in the centre of each plot (Chapter 7). 50ml of either methanol or *Phacelia* extract was placed in the dispenser. Ten wheat plants in a transect from one corner of the plots to the opposite corner were individually marked using electricians tape on the leaves of the plants. The pitfall traps were emptied weekly and the semiochemicals in the dispensers replaced with fresh material. Ten sweep net samples were taken at random from the plots each week, and the ten marked plants examined visually for the presence of adults or larvae of predators and pest species.

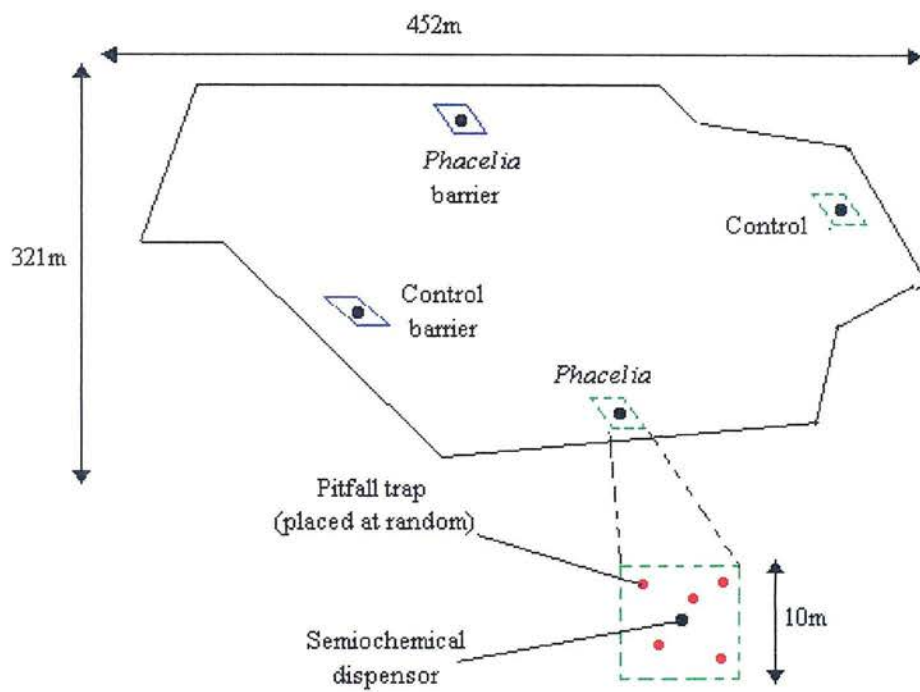


Fig. 8.2 The layout of the fields in 1998

8.2.2 Statistical analysis of field data.

The numbers of both active beetles and aphids per plot was modelled using a generalised linear model (Edgington & Gore 1986; Edgington, 1996) in the Genstat 5 statistics package (Copyright 1995, Lawes Agricultural Trust- Rothamsted Experimental station, Table 8.1). The model used was basically the same as the model used to analyse the field data from 1997 (Chapter 7.2.3 and Table 7.1). However the model was modified slightly as distance was not a factor in the 1998 field season. The Model had a log link ratio function and negative binomial distribution, which was judged by the residual mean deviance to fit the data better than a Poisson distribution. Field was regarded as a block factor, and treatment and week were regarded as treatment factors. A series of progressively more complex models were fitted and the mean deviances calculated. These were then compared to the residual mean deviance to give a variance ratio test and then overall probability obtained was from statistical tables.

Predicted means were calculated from the parameters of the model, however standard errors were increased to allow for a residual mean deviance of 1.298 rather than 1.

8.2.3 Enzyme linked immunosorbent assays (ELISAs)

Only gut contents from *P. melanarius* and *N. brevicollis* were used in the enzyme linked immunosorbant assays (ELISAs), as these were the most abundant predators in the fields. Beetles collected from the field from all three field seasons were taken back to the laboratory and frozen as soon as possible to preserve them for use in the

Table 8.1 Example of the genstat program for the analysis of field data

job	
open name='data98';channel=2	Inputs the data into the model in the correct form so subsequent analysis can take place.
factor sweek,sfield	
read [channel=2] sweek,sfield,v[1...4];frep=lev,lab,lev,4(*)	Tabulates the data into week and field and displays the tables
tabu [print=counts;class=sweek]	
&	
factor [class=sfield]	
factor [nvalues=192;labels=!t(Cont,Pha,Cont_Bar,Pha_Bar); values=48(1...4) treat	
factor [nvalues=192;levels=16] week	
factor [nvalues=192;levels=3] field	
equate old=!p((sweek)4);new=week	Sets the factors and creates the labels for the data enabling the model to read the data correctly.
&	
old=!p((sfield)4);new=field	
&	
old=v;new=nbeetles	
print week,field,treat,nbeetles;dec=0;fieldw=8	Displays the data for the identification of incorrectly entered values
model [dist=neg;disp=*] nbeetles	
fit field+week*treat	
predict [print=d,p,s] field	Sets the parameters of the model and fits a series of progressively more complex factors.
&	
week	
&	
treat	
&	
week,treat	
endjob	

ELISAs. The gut contents were first examined for the presence of slug protein as slug antibodies were available, whereas aphid antibodies were still underdevelopment. ELISAs were carried out according to a standard method (Symondson & Liddel, 1996).

The beetles were allowed to thaw out, and then dissected by firmly holding the beetles' abdomen between the thumb and forefinger of one hand and gently pulling away the head. The beetle's digestive tract remained attached to the head and pulls away from the rest of the body. The gut was then weighed and placed in a 1 ml microcentrifuge tube (Griener Laboritechnik, Kremenünster, Austria). Phosphate buffered saline (PBS, 8g NaCl, 0.2g KCl, 1.15g Na₂HPO₄ and 0.2g KH₂ PO₄ in 1L distilled water, Sigma Aldrich Chemical Co Ltd, Gillingham, UK) was added to the tube in a ratio of 1 : 20 weight of insect guts: volume of buffer. The beetle guts were then gently teased apart using a pair of forceps to help release the gut contents into the buffer. The tube was then spun in a centrifuge (Sigma 202 MK) at 12000 rpm for 5 minutes. This ensured an even distribution of the insect gut contents in the buffer and produced a 1:20 dilution of the gut contents. This was then further diluted by adding 1µl of diluted gut contents into 1ml of PBS and mixed thoroughly in a vortex mixer (Whirlimixer, Fisons Scientific Equipment, Loughborough, UK) producing a 1:20000 dilution of the gut contents.

S. avenae were weighed and added to PBS in a ratio of 1:20 weight of aphids : volume PBS. The mixture was then homogenised (T25 hermogenisor, Janke and Kunkel GMBH and Co, IKA laboritechnik, Germany). The mixture was then transferred into 1ml microcentrifuge tubes and centrifuged for 5 minutes at 12000

rpm to separate the liquid fraction from the solid fraction. The liquid fraction was retained by decanting into a clean microcentrifuge tube, to produce the aphid stock solution. This was then diluted by adding 1µl of stock aphid solution into 1ml of PBS to produce the aphid standard solution. This was repeated with *D. reticulatum*, the grey field slug to provide the slug standard. *S. avenae* and *D. reticulatum* were chosen for this because they are very common pests of cereal crops in south east Scotland.

The total protein content of the aphid and slug standard solutions was assessed using a commercial protein determination kit (Sigma-Aldrich chemical Company Ltd, Gillingham, UK).

A dilution series of the standard solutions consisting of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, 0.39% and 0% standard solution was prepared using a 1:20000 dilution of beetle gut, fed solely on earthworm, in PBS. This provided the non reactive worm control, which ensured that there is no cross-reaction of the antibody with other foreign proteins.

The dilution series of aphid standard solution was then placed in the first two rows of a microtitre plate, 100µl of each of the dilutions was placed into the wells with 2 replicates, starting with the most concentrated at the right hand side of the plate and most dilute at the left hand side. The last four wells were filled with 100µl of PBS which act as blanks. The rest of the wells on the plate were filled with 1:20000 dilution of beetle guts samples with 2 replicates of each sample (Fig 8.3). The plates were then left for 24 hours at room temperature to allow the proteins to anneal to the walls of the wells.

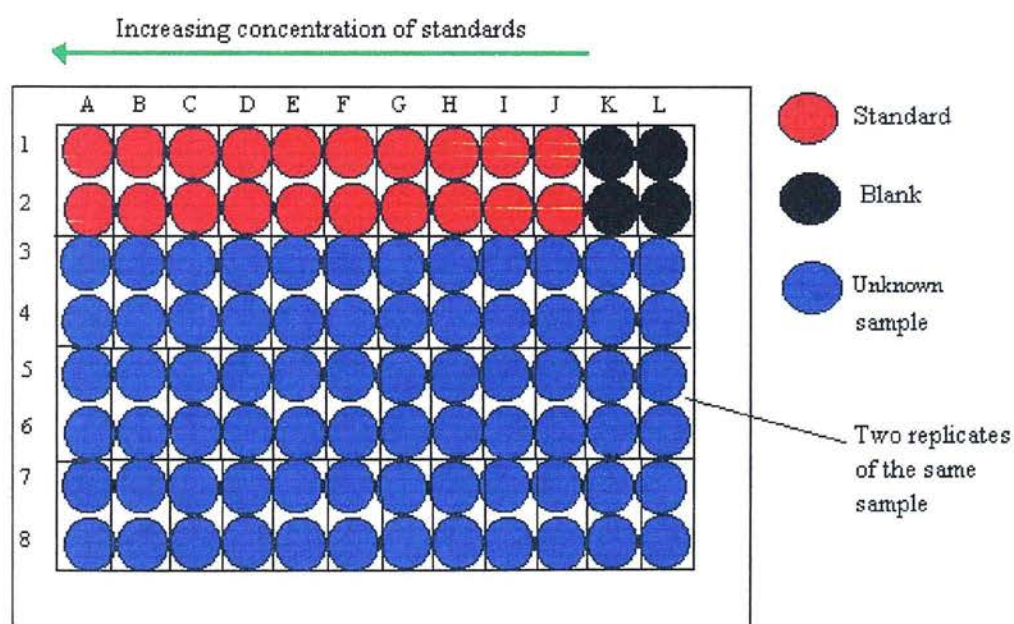


Fig 8.3 Diagram of the ELISA plate

The plates were washed 3 times in PBS Tween (250µl Tween 20 in 500ml PBS, Sigma Aldrich Chemical Company Ltd, Gillingham, UK) using a plate washer (Ultrawash II, Dynatech Medical Products, Guernsey, UK). The wells were filled with 100µl of bovine serum albumen (BSA), a stable non reactive protein that takes up any site in the well that is not occupied by insect gut proteins, and prevent any other protein binding there later in the assay, such as the antibodies, which may lead to false positive results. However, being non-reactive, BSA will also not bind with the antibodies. The plate was left for 1 hour at room temperature to allow the BSA to anneal. A 1:20000 dilution of mouse IgG anti-aphid monoclonal antibody (School of Biosciences, University of Cardiff, UK) was prepared in PBS Tween. Tween 20 increased the specificity of the reaction by preventing binding of none specific proteins. The plate was again washed three times to remove excess BSA. 100µl of diluted antibody was placed into each well of the plate. The plate was then left for 2 hours to allow the antibodies to bind to the aphid proteins.

A 1: 5000 dilution of rabbit antimouse IgG/ peroxidase conjugate (Sigma-Aldrich Chemicals Ltd, Gillingham, UK) was prepared in PBS Tween. The plate was washed again three times to remove excess antibody. The wells on the plate were left for 1 hour at room temperature to enable the conjugate to bind to the antibody.

The plate was washed 3 times to remove excess conjugate. One o-phenylenediamine (Sigma-Aldrich Chemical Company Ltd, Gillingham, UK) tablet was added to 50ml of citrate phosphate buffer to produce the substrate. Just before the substrate was added to the plate, 0.5ml of hydrogen peroxide was added. This has to be left until

the last moment as it produced a photosensitive reaction in the substrate which begins to change colour when exposed to light. 100 µl of substrate was added to each well as quickly as possible. The plate was left for 30 minutes in a light proof cupboard. The peroxidase conjugate catalysed the reaction in the dark producing a colour change in wells which contain aphid proteins. The experiment was repeated using mouse antislug IgG (School of Biosciences, University of Cardiff, UK).

The plates were read using a plate reader (Multiscan RC, Labsystems, Helsinki, Finland) attached to an IBM PC, and the data were analysed by the Genesis software package (Labsystems, Helsinki, Finland).

8.2.4 Statistical analysis of ELISA data

Large variations in prey protein content in individual beetle's guts and in the number of active beetles trapped in each plot meant that analysis of variance was inappropriate in relation to ELISA data. Therefore, these data were analysed by generalised linear modelling using the Genstat 5 statistics package (Copyright 1995, Lawes Agricultural Trust- Rothamsted Experimental Station). Because of the large variations in both beetle numbers and prey protein content, the data was converted into presence/absence data. In order to determine if prey protein was present in the gut contents, it was necessary to set a threshold concentration above which the beetle would be prey protein positive. The threshold concentration of prey protein in the beetle guts was set at a haemolymph concentration equivalent (HCE) of 91 nl/ml.

Table 8.2 Genstat linear model for analysis of ELISA data

```

job
open name='PAPH97.gdt';channel=4;width=160
factor [labels=!t('0-10','45-55','90-100')] sdist
factor sweek,sfield
read [channel=4] sweek,sdist,sfield,v[1...12];frep=lev,lab,lev,6(*)
tabu [print=counts;class=sweek]
& [class=sdist]
& [class=sfield]
variate [values=1...702] n
unit n
factor [nvalues=702;labels=!t(Control,Spray_Co,Phacelia,Nepeta,'(E)-B-F',\
  Envirofeat); values=117(1...6)] treat
factor [nvalues=702;levels=13] week
factor [nvalues=702;levels=3] field
factor [nvalues=702;levels=3;labels=!t('1.-10','45-55','90-100')] dist
variate [nvalues=702] naphid,nbeetles
equate old=!p((sweek)6);new=week
& old=!p((sdist)6);new=dist
& old=!p((sfield)6);new=field
& old=!p(v[1,3,5,7,9,11]);new=naphid
& old=!p(v[2,4,6,8,10,12]);new=nbeetles
print n,week,dist,field,treat,naphid,nbeetles;dec=0;fieldw=8
model [dist=bin;disp=*] naphid;nbin=nbeetles
fit [factorial=2] field
& field+dist
& field+dist+treat
& field+dist*treat
& field+dist*treat+week
predict [print=d,p,s;alias=ignore] field
& dist
& week
& treat
endjob

```

Inputs the data into the model in the correct form so subsequent analysis can take place.

Tabulates the data into week distance and field and displays the tables

Displays the data for the identification of incorrectly entered values.

Sets the parameters of the model and fits a series of progressively more complex factors.

Generates and displays the predictions.

HCE is the concentration of unknown sample that gives an optical density equivalent to 91nl of prey haemolymph per ml of sample in the spectrophotometer. The threshold concentration was set at 91nl/ml as this was the lowest concentration of the pest haemolymph in the standards at 1:20000 dilution (Symondson & Liddell, 1993). The data were analysed by a generalised linear model (Edgington & Gore, 1986; Edgington, 1995, Table 8.2), which was modified to accept presence absence data in the form of number of positive beetles and the total number of beetles per sample. Presence/absence data has a binomial distribution, therefore the model was modified to binomial variation with a logistic link function rather than negative binomial variation with a log-link ratio function used in the field trials (Chapter 7.2.3, Table 7.1).

Field was regarded as a block factor while treatment, distance and week were regarded as treatment factors. A series of progressively more complicated models were fitted and the mean deviances calculated. The mean deviances were then compared to the residual mean deviance in a variance ratio test. Predicted means were calculated from the parameters of the model.

8.3 Results

8.3.1 Field studies 1998

There was a significant increase in the number of carabid beetles present in the non-barriered plots with *Phacelia* extract relative to the methanol control plots ($P < 0.001$ Fig. 8.4). However, there was no significant difference between the number of active

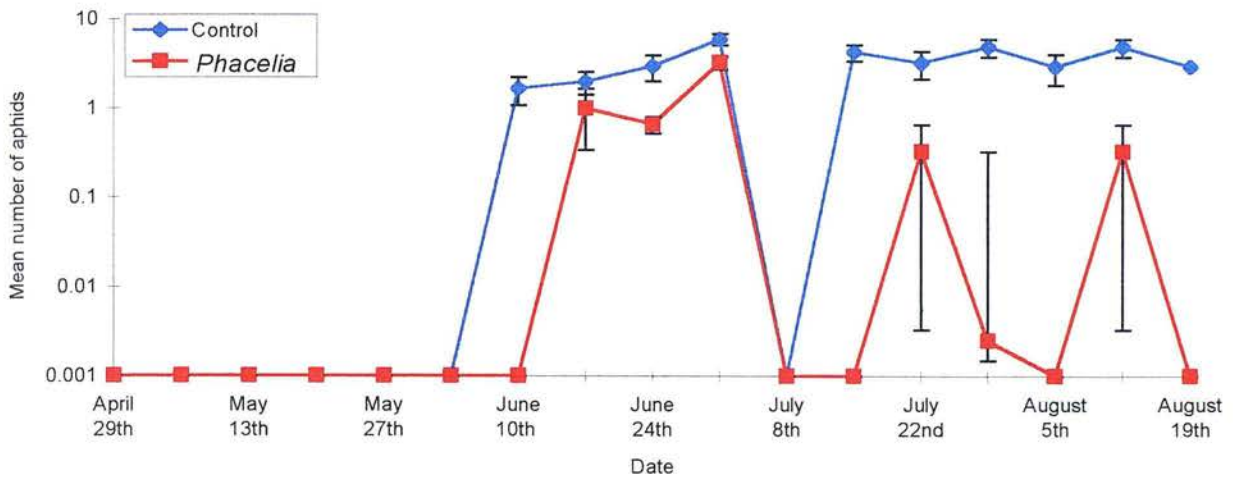


Fig. 8.4 Mean number (\pm SE) of carabids trapped in the *Phacelia* flower extract treated non-barriered plots relative to control non-barriered plots (sample size=3).

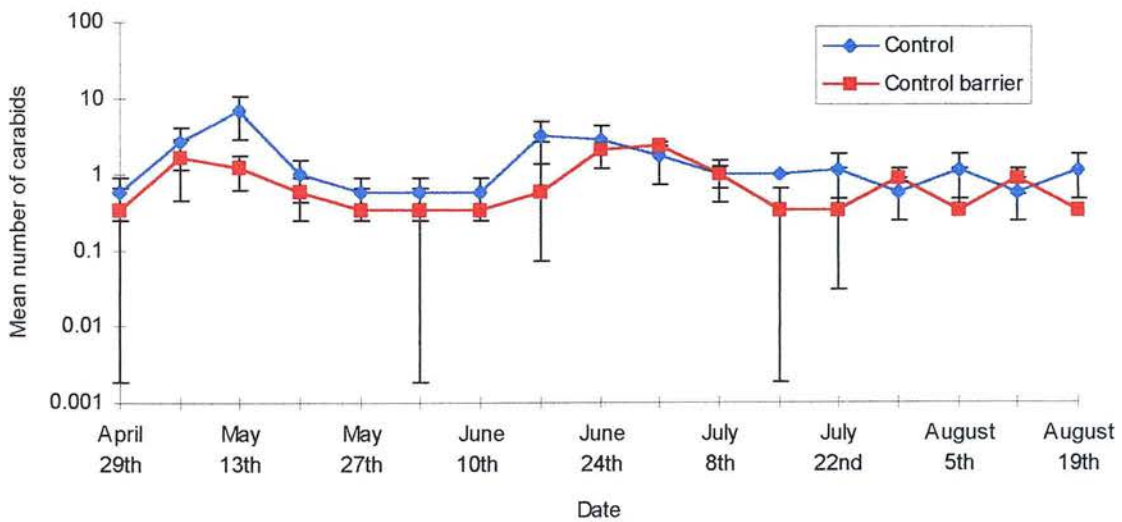


Fig. 8.5 Mean number (\pm SE) of carabids trapped in controlled barriered plots relative to the control non-barriered plots (sample size=3).

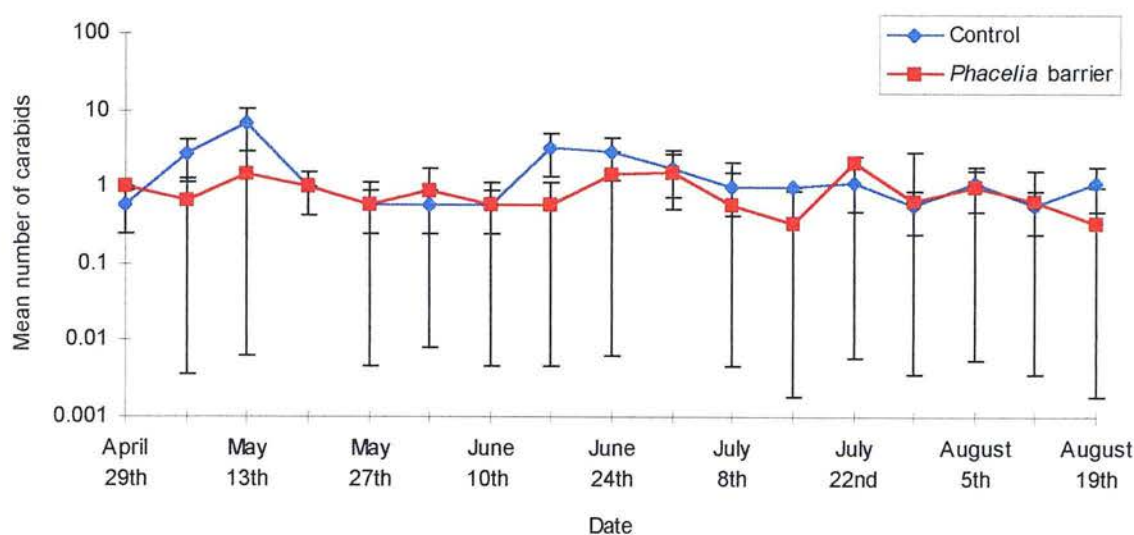


Fig. 8.6 Mean number (\pm SE) of carabids trapped in the *Phacelia* barriered relative to control non-barriered plots (sample size=3)

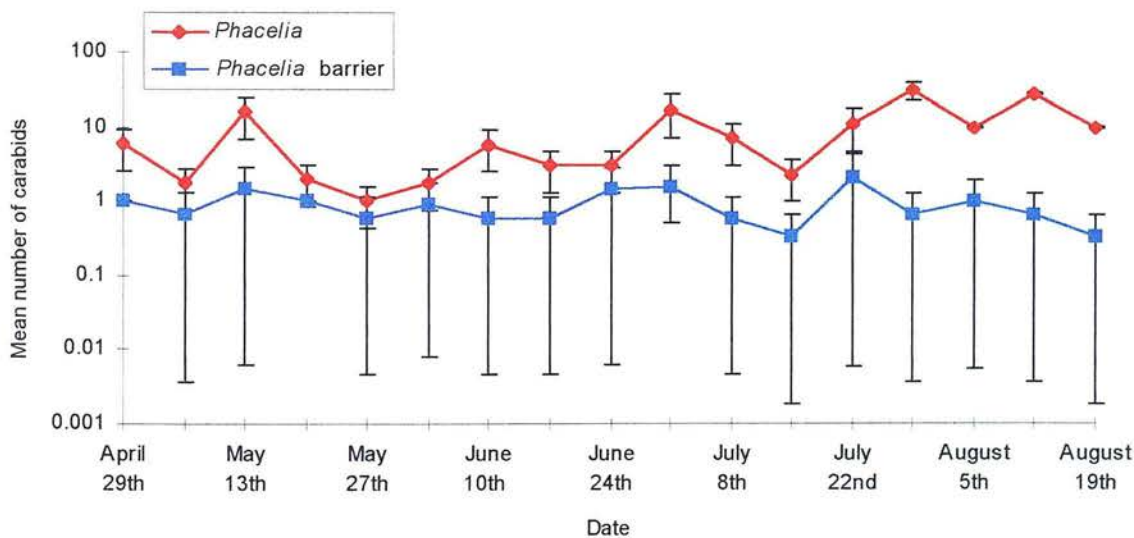


Fig. 8.7 Mean number (\pm SE) of carabids trapped in the *Phacelia* barriered relative to non-barriered plots (sample size=3)

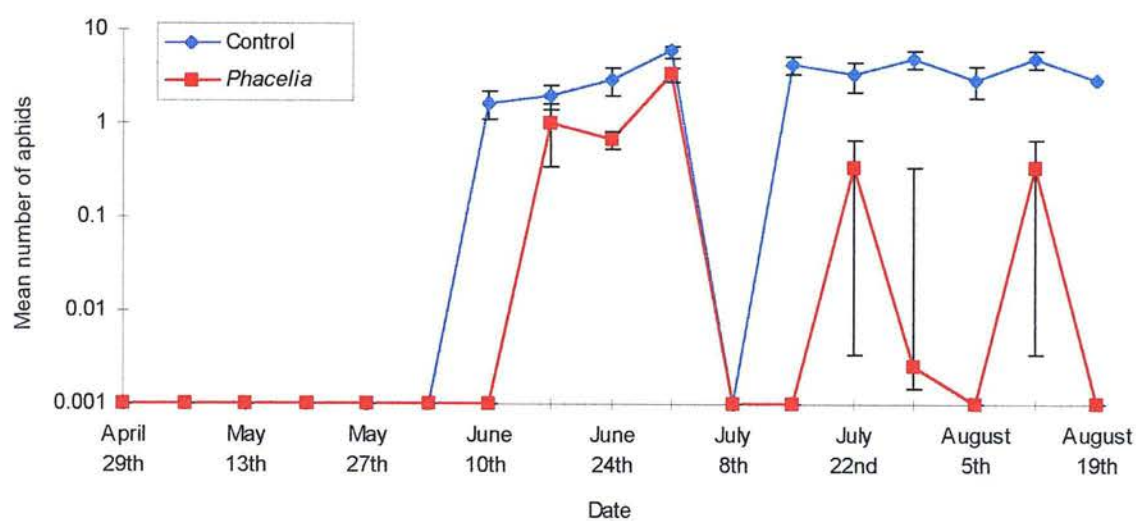


Fig. 8.8 Mean number (\pm SE) of aphids trapped in *Phacelia* flower extract treated non barriered plots relative to control non-barriered plots (sample size=3).

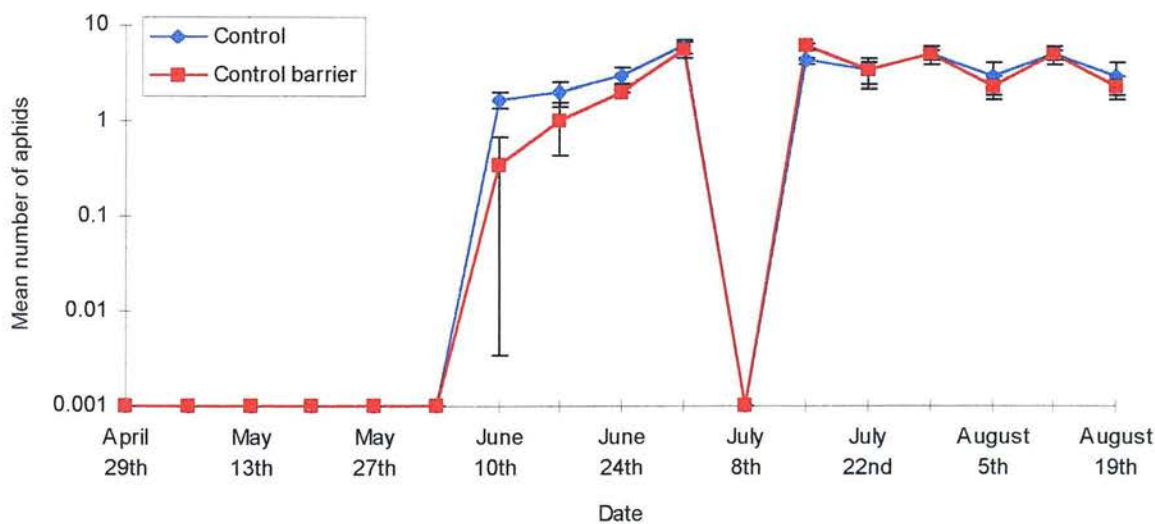


Fig. 8.9 Mean number (\pm SE) of aphids trapped in controlled barriered plots relative to the control non-barriered plots (sample size=3).

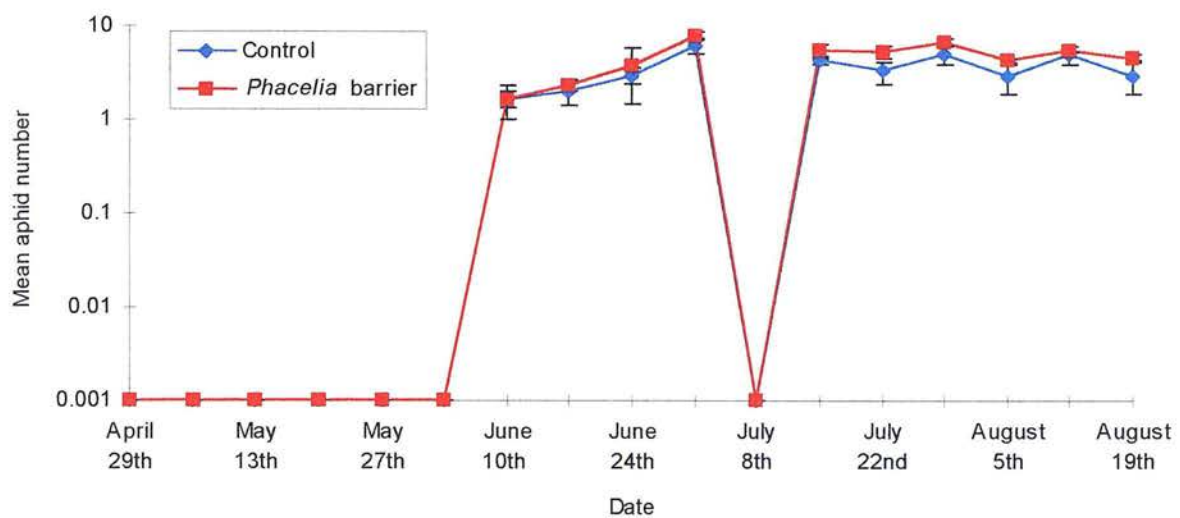


Fig. 8.10 Mean number (\pm SE) of aphids trapped in the *Phacelia* barriered relative to control non-barriered plots (sample size=3)

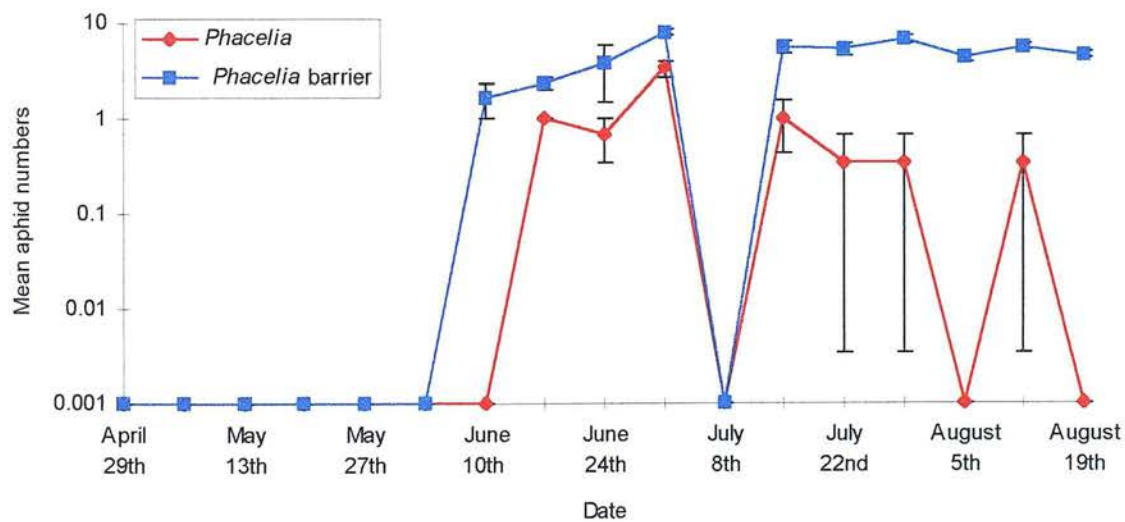


Fig. 8.11 Mean number (\pm SE) of aphids trapped in the *Phacelia* barriered relative to non-barriered plots (sample size=3).

carabids trapped in the control plots, the control barriered plots and the *Phacelia* barriered plots ($P>0.05$, Figs. 8.5-6). There were significantly fewer carabids in the *Phacelia* barriered plots relative to the *Phacelia* non-barriered plots ($P<0.001$, Fig. 8.7).

There were significantly fewer aphids in the *Phacelia* plots relative to the control plots ($P<0.001$, Fig. 8.8). There was no significant difference in the number aphids in the control barriered plots relative to the control non-barriered plots ($P>0.05$, Fig. 8.9). There was a small but significant increase in the number of aphids in the *Phacelia* barriered plots relative to the control non-barriered plots ($P<0.01$, Fig. 8.10). There was a significant increase in the number of aphids in the *Phacelia* barriered plots relative to the *Phacelia* non-barriered plots ($P<0.001$, Fig. 8.11). P values for the 1998 field season for carabids and aphids are shown in Appendix 6, Table 10.15-16.

8.3.2 ELISA tests of carabid gut contents.

In 1996 large numbers of beetles were found which had equal to or above the threshold concentration of 91 nl/ml, aphid or slug protein in their gut contents. However, neither field (for aphid and slug protein with *P. melanarius* and *N. brevicollis* $P>0.05$, Figs. 8.12 and 8.15), or treatment (for aphid and slug protein with *P. melanarius* and *N. brevicollis* $P>0.05$, Fig. 8.13 and 8.16), had a significant effect on the percentage of beetles trapped with significant level of prey protein in their guts. There were significant changes in the percentage of beetles trapped with ≥ 91 nl/ml throughout the time course of the experiment (for aphid

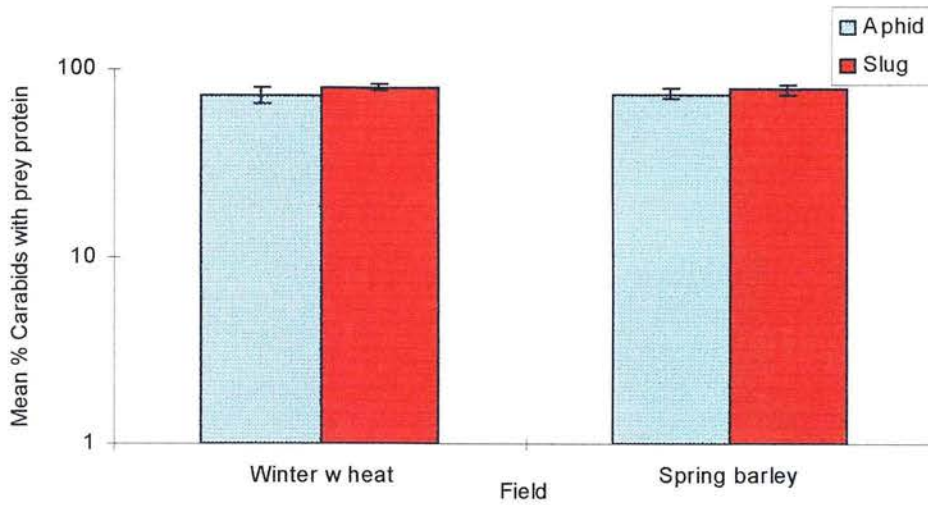


Fig. 8.12 Mean percentage (\pm SE) of *P. melanarius* trapped from each field in 1996 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

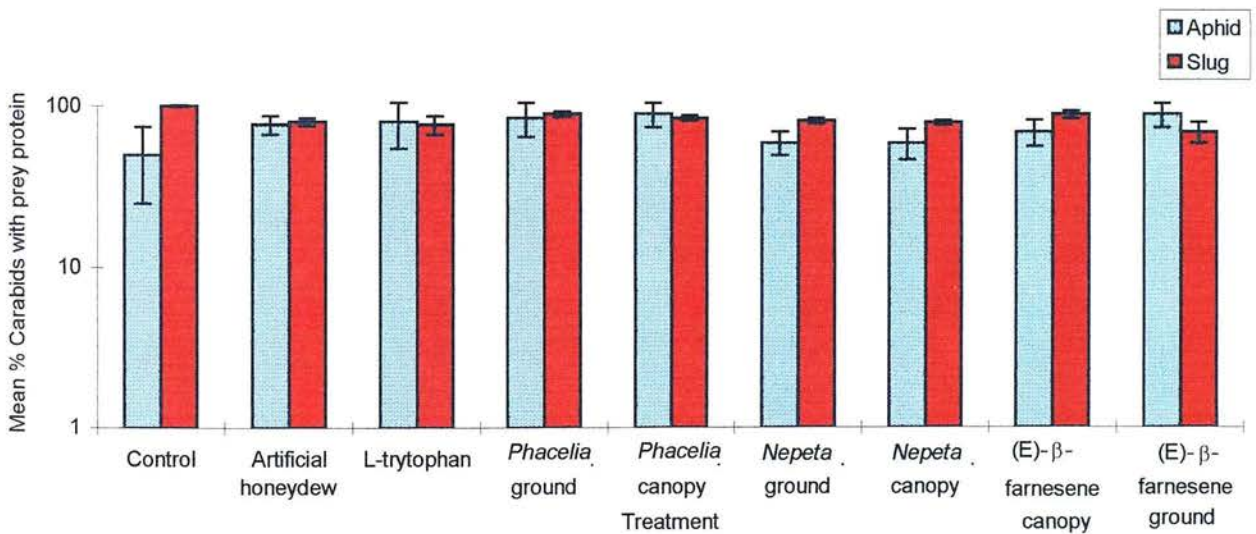


Fig. 8.13 Mean percentage (\pm SE) of *P. melanarius* trapped from each treatment in 1996 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

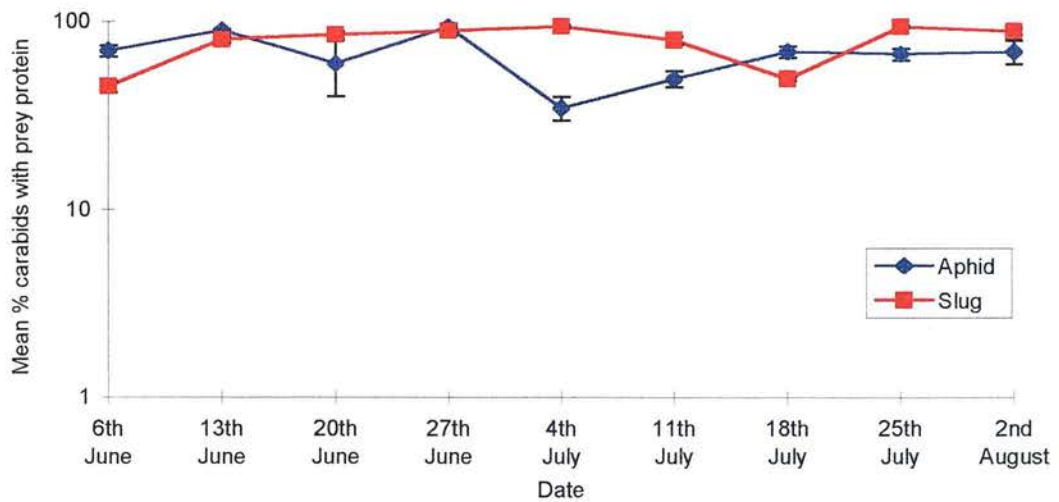


Fig. 8.14 Mean percentage (\pm SE) of *P. melanarius* trapped each week in 1996 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

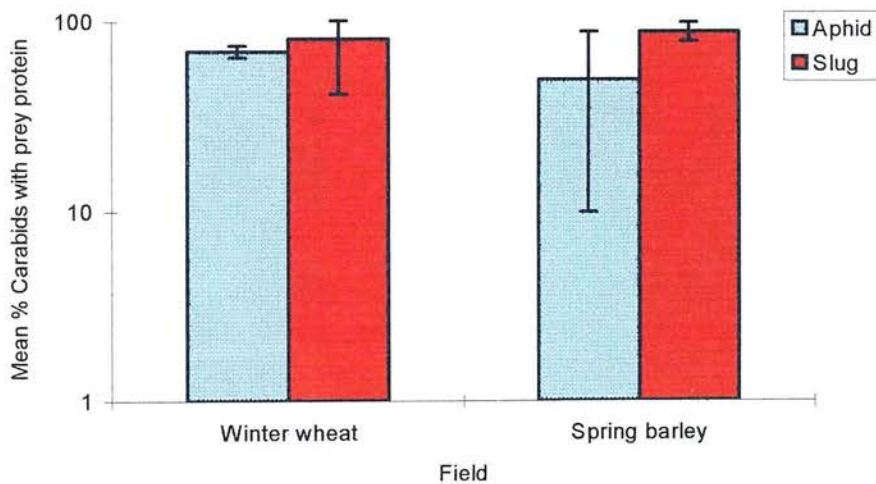


Fig. 8.15 Mean percentage (\pm SE) of *N. brevicollis* trapped from each field in 1996 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

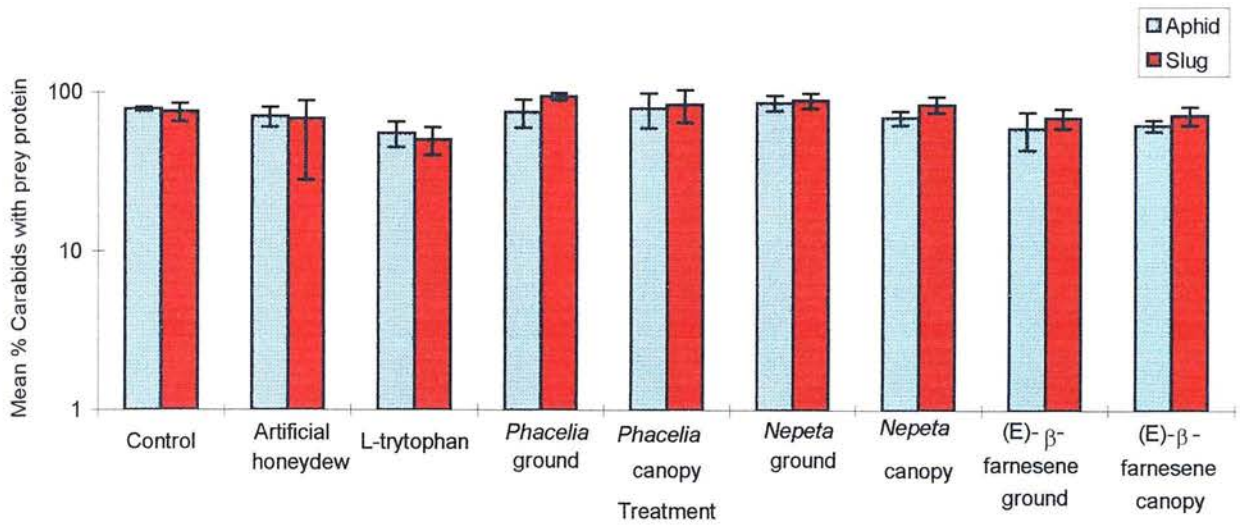


Fig. 8.16 Mean percentage (± SE) of *N. brevicollis* trapped from each treatment in 1996 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

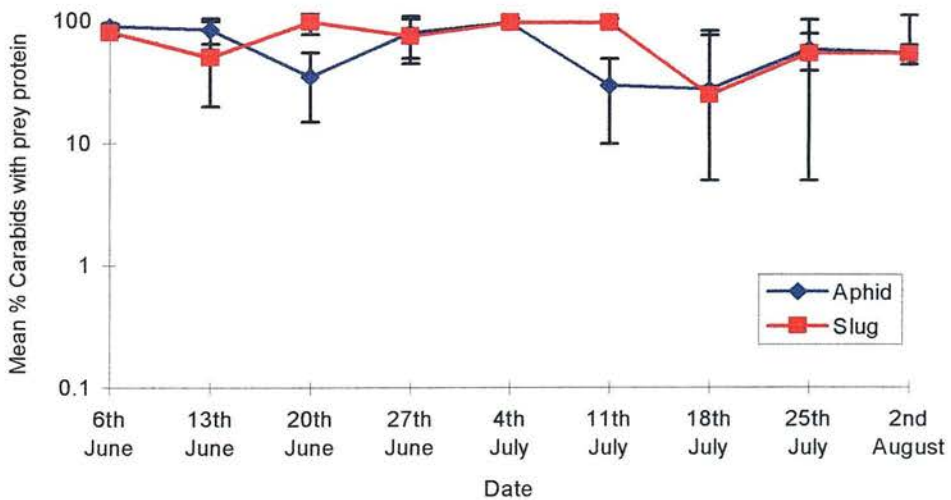


Fig. 8.17 Mean percentage (± SE) of *N. brevicollis* trapped from each week in 1996 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

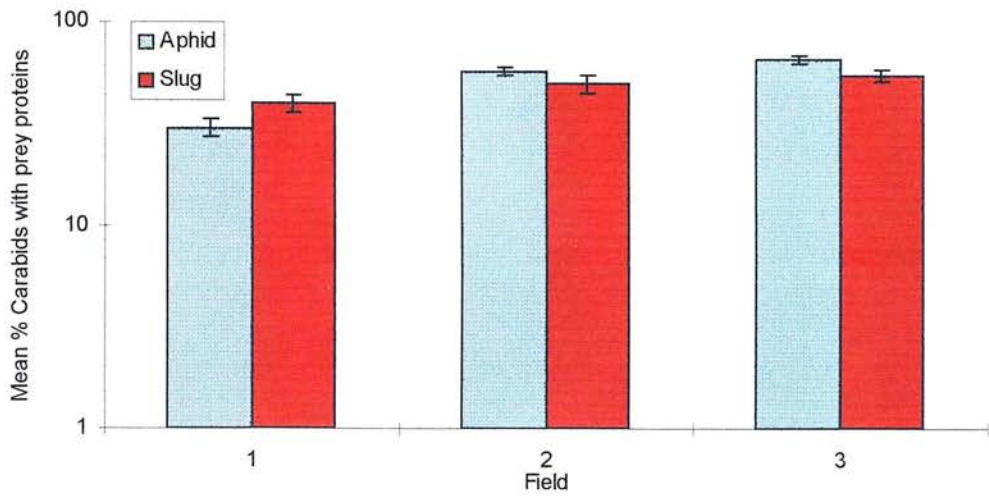


Fig. 8.18 Mean percentage (\pm SE) of *P. melanarius* trapped from each field in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

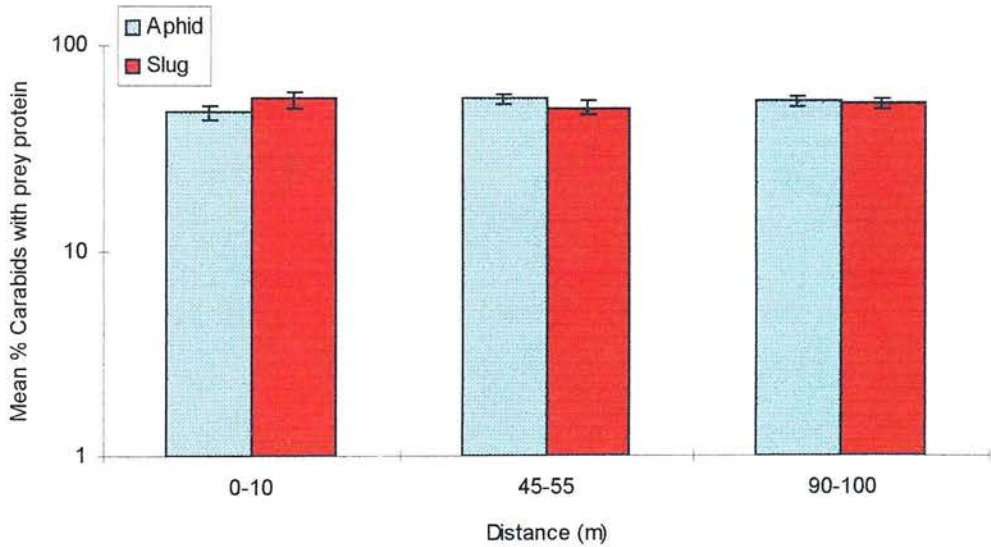


Fig. 8.19 Mean percentage (\pm SE) of *P. melanarius* trapped from each distance in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

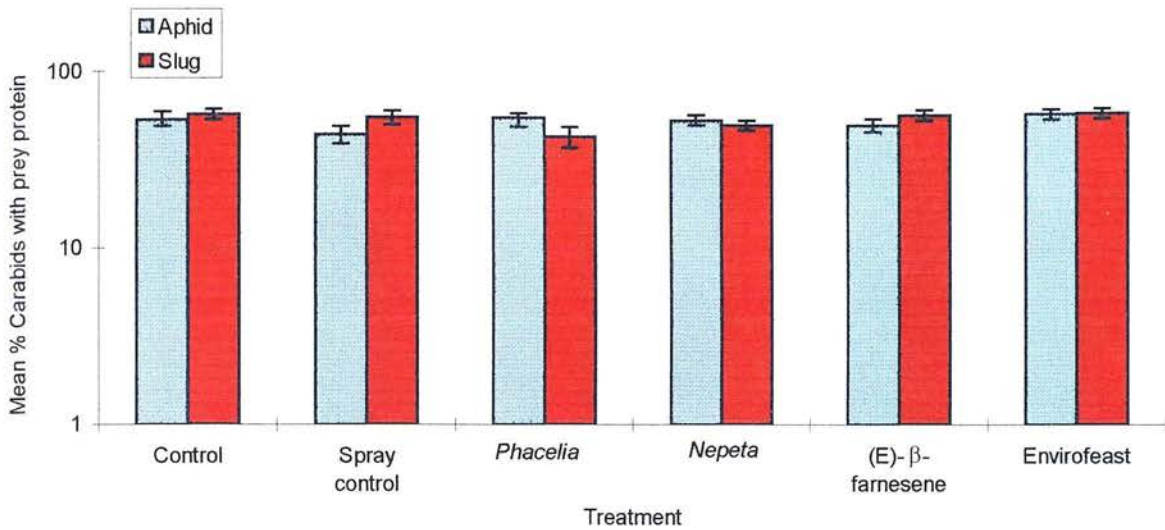


Fig. 8.20 Mean percentage (\pm SE) of *P. melanarius* trapped from each treatment in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

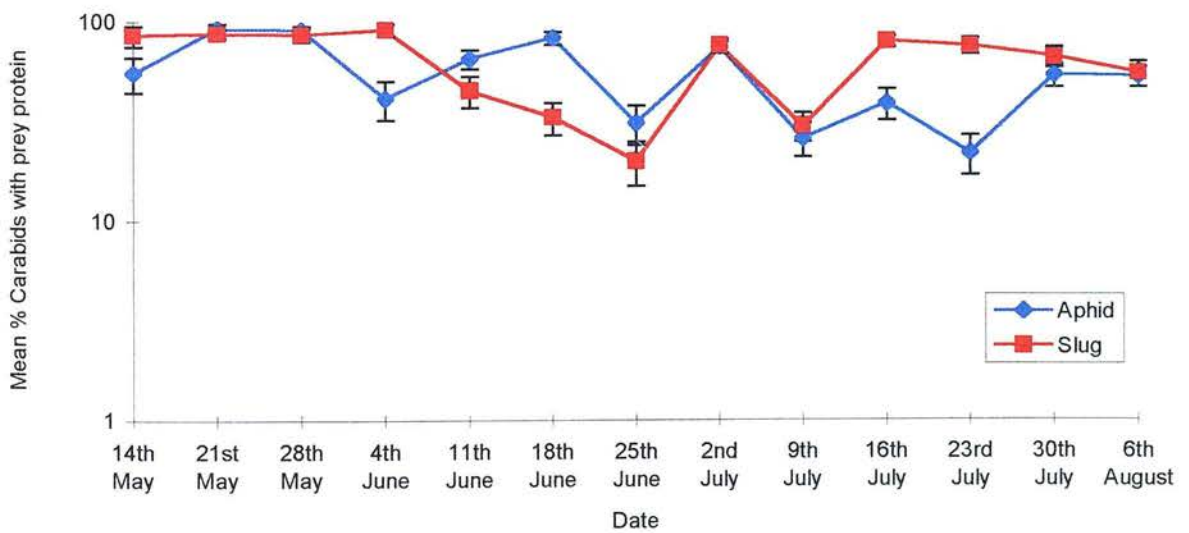


Fig. 8.21 Mean percentage (\pm SE) of *P. melanarius* trapped from each week in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

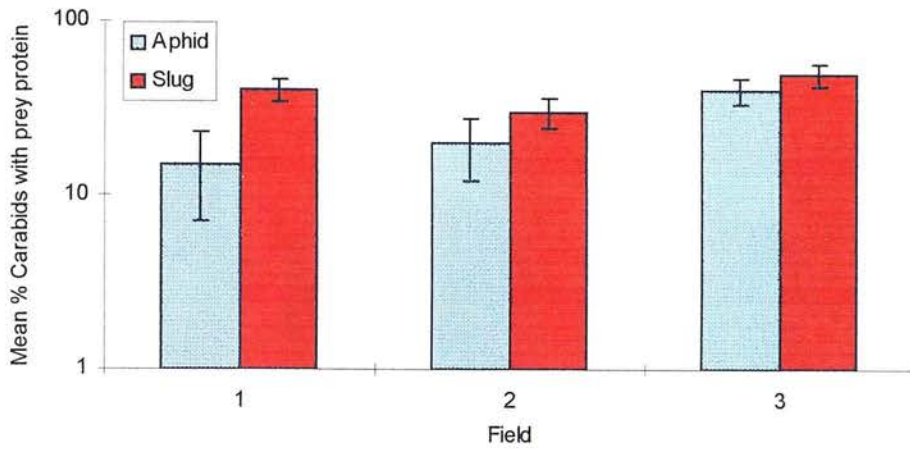


Fig. 8.22 Mean percentage (\pm SE) of *N. brevicollis* trapped from each distance in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

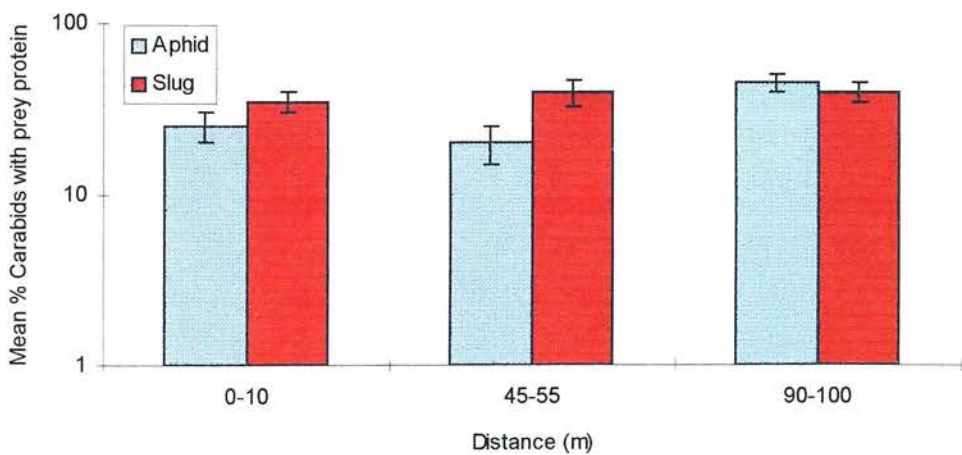


Fig. 8.23 Mean percentage (\pm SE) of *N. brevicollis* trapped from each field in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

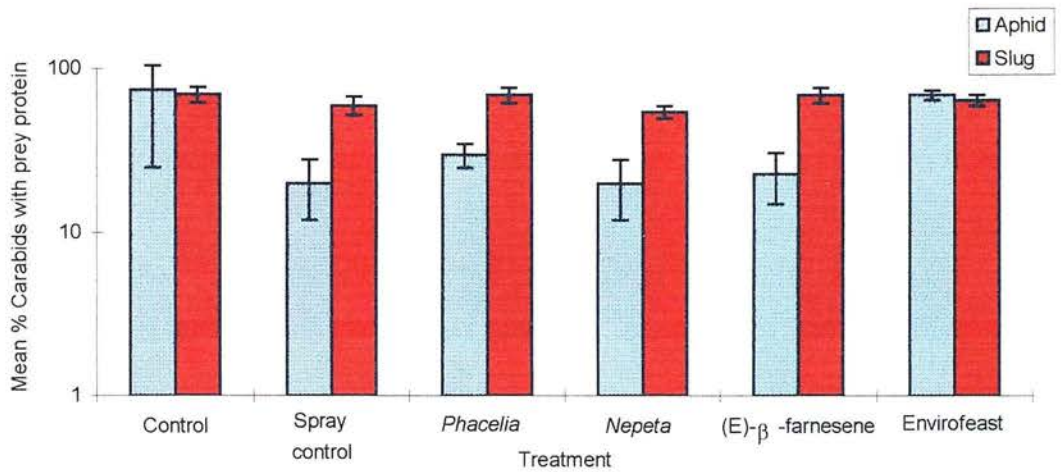


Fig. 8.24 Mean percentage (\pm SE) of *N. brevicollis* trapped from each treatments in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

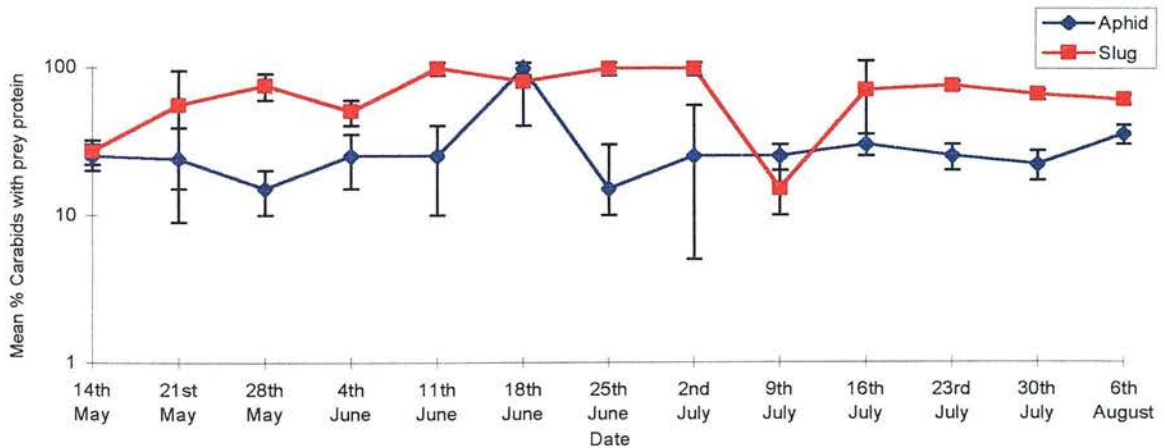


Fig. 8.25 Mean percentage (\pm SE) of *N. brevicollis* trapped from each week in 1997 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

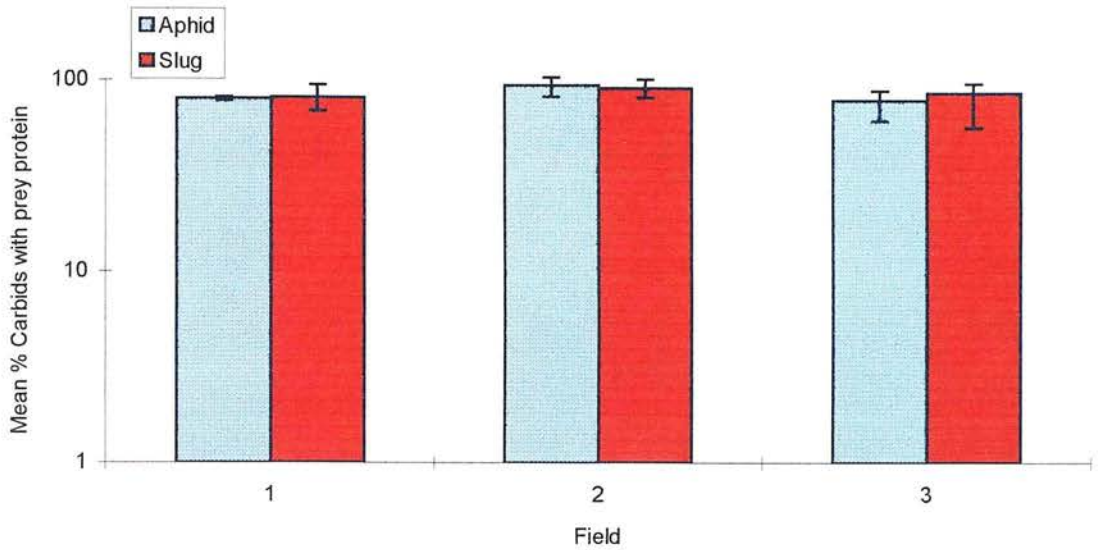


Fig. 8.26 Mean percentage (\pm SE) of *P. melanarius* trapped from each field in 1998 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

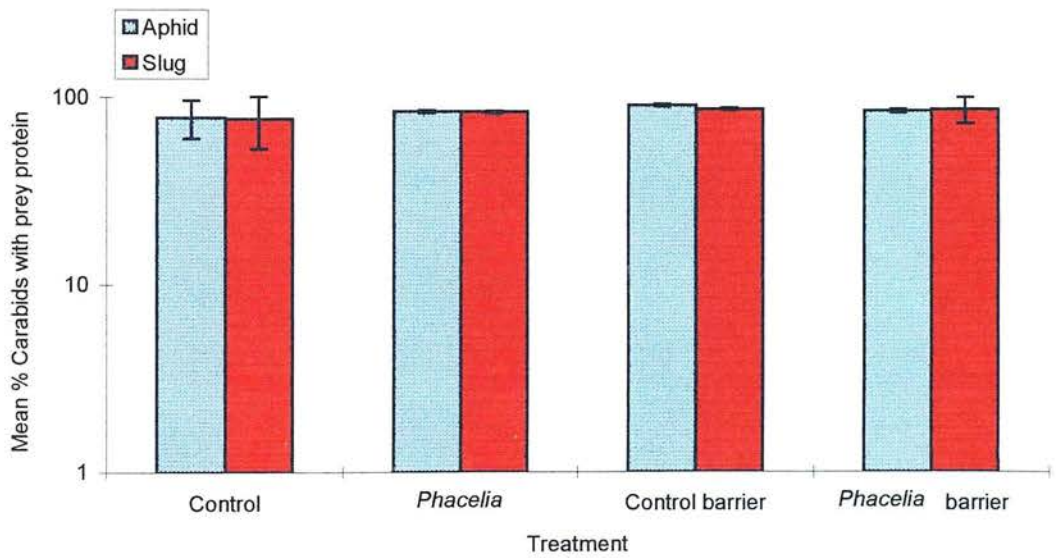


Fig. 8.27 Mean percentage (\pm SE) of *P. melanarius* trapped from each treatment in 1998 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

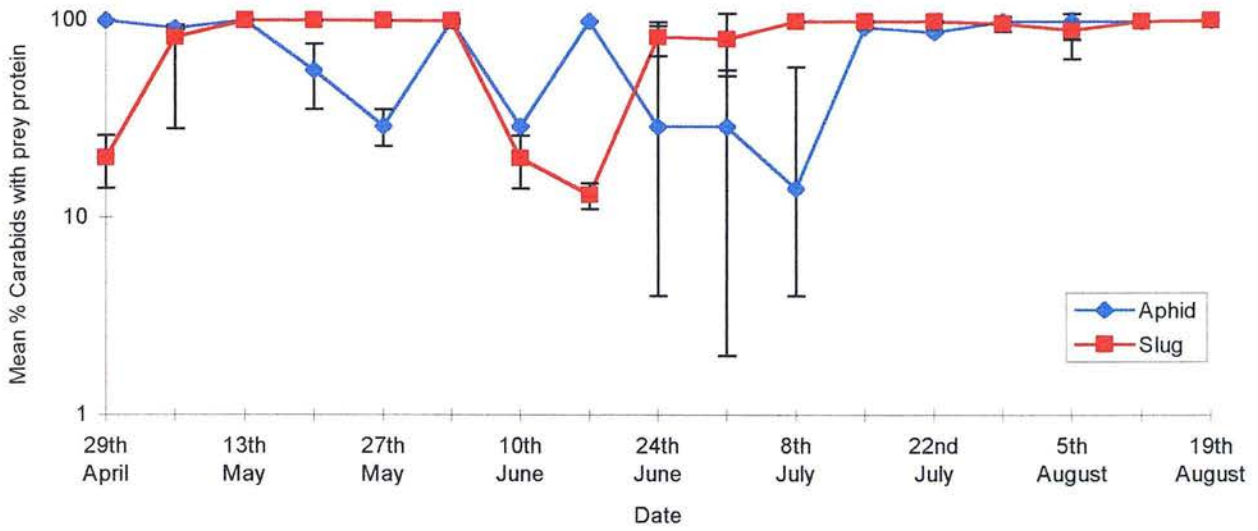


Fig. 8.28 Mean percentage (\pm SE) of *P. melanarius* trapped from each week in 1998 with significant ($\geq 100 \mu\text{l}/\text{ml}^{-1}$) levels of prey proteins in their guts (sample size=3).

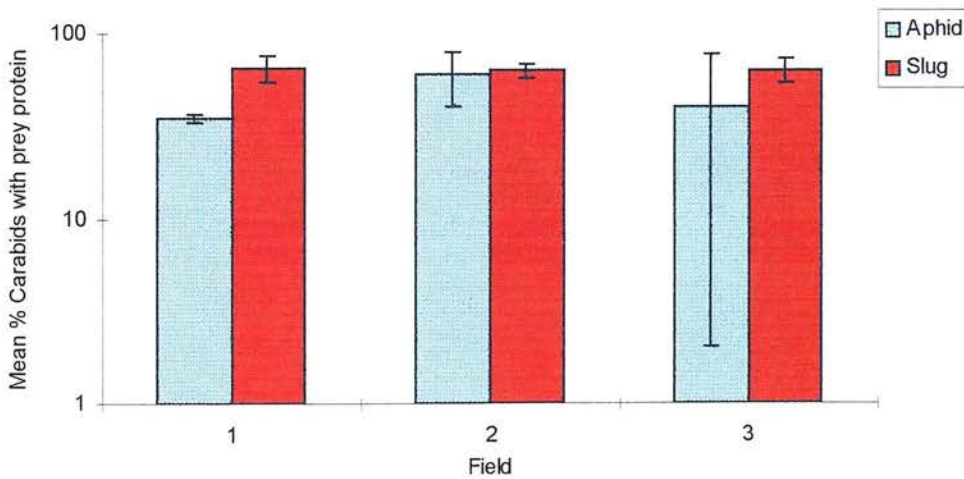


Fig. 8.29 Mean percentage (\pm SE) of *N. brevicollis* trapped from each field in 1998 with significant ($\geq 91 \text{ nl}/\text{ml}$) levels of prey proteins in their guts (sample size=3).

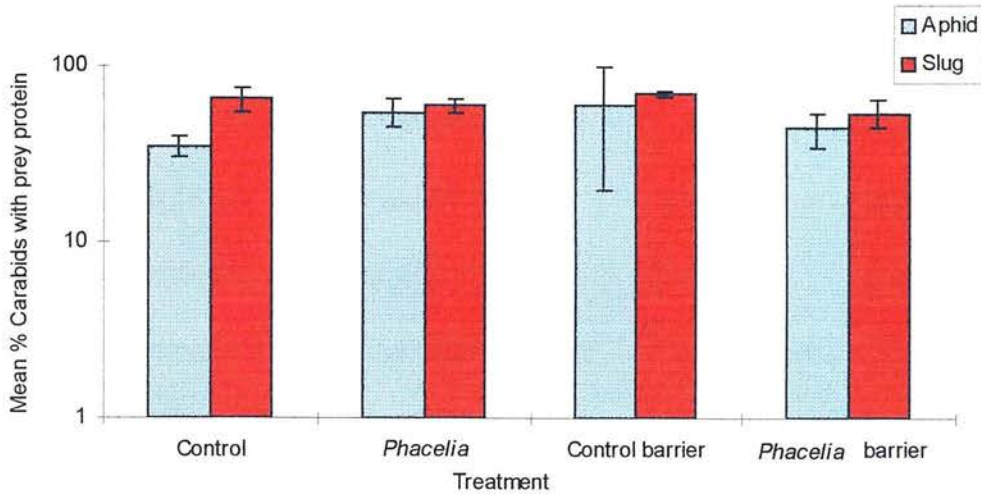


Fig. 8.30 Mean percentage (\pm SE) of *N. brevicollis* trapped from each treatment in 1998 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

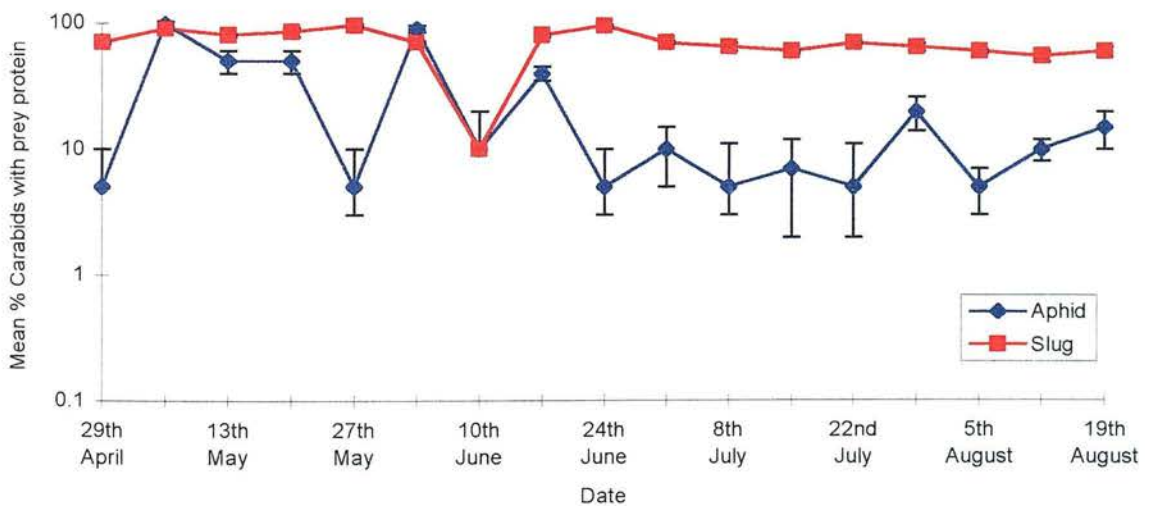


Fig. 8.31 Mean percentage (\pm SE) of *N. brevicollis* trapped from each week in 1998 with significant (≥ 91 nl/ml) levels of prey proteins in their guts (sample size=3).

protein: *P. melanarius* $P < 0.001$, *N. brevicollis* $P < 0.01$; slug protein: *P. melanarius* $P < 0.01$, *N. brevicollis* $P < 0.001$, Fig. 8.14 and 8.17). In 1997 a similar pattern emerges with field (for aphid and slug protein in *P. melanarius* and *N. brevicollis* $P > 0.05$, Figs. 8.18 and 8.22), distance from the field edge (for aphid and slug protein in *P. melanarius* and *N. brevicollis* $P > 0.05$, Figs. 8.19 and 8.23), or treatment (for aphid and slug protein in *P. melanarius* and *N. brevicollis* $P > 0.05$, Figs. 8.20 and 8.24), having no significant effect on the percentage of beetles trapped which contain 91 nl/ml or more of prey protein in their gut contents. However, there were significant changes in the proportion of beetles containing ≥ 91 nl/ml of prey protein in their gut contents from week to week in the experiment (for aphid protein: *P. melanarius* $P < 0.001$, *N. brevicollis* $P < 0.001$; slug protein: *P. melanarius* $P < 0.001$, *N. brevicollis* $P < 0.001$, Figs. 8.21 and 8.25).

In 1998 neither field (for aphid and slug protein in *P. melanarius* and *N. brevicollis* $P > 0.05$, Figs. 8.26 and 8.29), or treatment (for aphid and slug protein in *P. melanarius* and *N. brevicollis* $P > 0.05$, Figs. 8.27 and 8.30), had a significant effect on the proportion of beetles containing 91 nl/ml or more of prey protein in their gut contents. However, there was a significant difference in prey protein gut contents over the weeks (for aphid protein: *P. melanarius* $P < 0.001$, *N. brevicollis* $P < 0.001$; slug protein: *P. melanarius* $P < 0.001$, *N. brevicollis* $P < 0.001$, Figs. 8.28 and 8.31). P values for the Elisa on predator gut contents are shown in Appendix 7, Table 10.17-22.

8.4 Discussion

8.4.1 Field studies 1998

As in the study of Holland & Thomas (1997), the barriers did not completely prevent the entry of carabids into the plots. However, the number of carabids in the barriered plots was significantly reduced, therefore barriers excluded most beetles from entering the plots particularly with the *Phacelia* barriered plots as compared to the *Phacelia* non-barriered plots. Barriers in this and in Holland & Thomas (1997) study greatly reduce the colonisation of the plots by carabids from outside. Pitfall traps in the barriered plots removed individual beetles which can only be replaced slowly (Holland & Thomas, 1997).

As in previous years studies (Chapter 7), there was a significant increase in the number of carabids present in the *Phacelia* extract treated non-barriered plots relative to both control plots and *Phacelia* barriered plots. This suggests that increased numbers of carabids were attracted in from outside the plot by the odour of *Phacelia*. This agrees with the findings of Kielty *et al* (1995), who found carabids were attracted to volatile wind borne semiochemicals, and Hickman & Wratten (1996), who found that *Phacelia* plants increased the numbers of predators in the area.

Aphid populations did not remain constant throughout the field season; aphid numbers started low and began to build up in the beginning of June. However, during the 1998 field season in south east Scotland there were adverse weather conditions, which may account for some of the variation in measured aphid populations. For example the severe decline in aphid numbers in the week of July 8th coincided with

an increase in the amount of precipitation from 2 mm in the previous week to 20mm, and a fall in average temperature from 19°C to 8°C.

There was a significant decrease in the number of aphids present in the *Phacelia* non-barriered plots relative to the control plots. However, there was no significant difference the numbers of aphids in the *Phacelia* barriered plots relative to the control plots. Carabids were excluded from the barriered plots and aphid numbers remained high, and this suggests that the *Phacelia* extract does not have a behavioural effect on the aphids themselves, if *Phacelia* affected aphid behaviour, aphid numbers would have differed in the *Phacelia* barriered plots. This suggests that the increased numbers of carabids in the plots are responsible for the reduction in aphid numbers. This supports the findings of Holland & Thomas (1997), who found that exclusion of natural populations of polyphagous carabids from plots in cereal fields resulted in approximately a 30% increase in aphid numbers. However, the 30% reduction in aphid populations caused by predation by natural populations of carabids was not enough to have an economic effect (Thomas & Holland, 1997). By enhancing natural populations of polyphagous predators through the use of attractive semiochemicals there is a greater impact on aphid populations which may be enough to produce economic benefits.

8.4.2 ELISA

The ELISA of beetle gut contents indicate that a significant proportion of the beetles trapped in all of the plots were feeding on both aphids and slugs, shown by the presence of both aphid and slug proteins in the gut contents of *P. melanarius* and *N.*

brevicollis at concentrations in excess of 91 nl/ml. Both treatment and field factors did not have a significant effect on the percentage of beetles in the plots which had these prey proteins in their guts. This may be because individual beetles exhibit a preference when given a choice of prey. Prey preference has been seen in a variety of different insect predators and may be due to a variety of factors such as physiological state, optimal diet, developmental diet, maternal diet, learning and preconditioning, and presence or absence of specific infochemical cues (Hazzad & Ferro, 1991; Failes, Whistlecraft & Tomlin, 1992; Dejong and Kaiser, 1992; Agelopolous, Dicke & Posthumus 1995; Dippel & Hilker, 1998). Prey and host preferences have been demonstrated in other studies involving monophagous predators (Mizuno, Itioka, Tatematsu and Ito, 1997), polyphagous predators (Hazzad & Ferro, 1991), and parasitoids (Kraaijeveld, Nowee & Najem, 1995). These findings agree with those of Bilde & Toft (1994; 1997) who found evidence for prey preference in carabids in the field. Approximately the same percentage of carabids in the entire population may exhibit a preference for slugs and aphids, therefore the percentage with slug and aphid proteins in their gut contents will not vary with treatment and field.

The only factor which did effect the percentage of beetles which had aphid and slug protein content in their guts was sampling date. This may be because prey numbers are not static throughout the experiment but do change over time, e.g. aphid abundance was low at the beginning of the season, and then begins to build up. If a prey item is not present then the beetles will not be able to eat it even if they prefer that particular food. Although polyphagous carabids could survive in the area either by feeding on another prey item (Hazard & Ferro, 1991), or by entering diapause

(Forsythe, 1987), the percentage of carabids with protein from that particular prey item in their guts would fall.

This is an advantage in the use of polyphagous predators as biological control agents, as they can be maintained in relatively high numbers on arable land by the use of semiochemicals even when numbers of pests are low, due to mechanisms to survive periods of prey shortage (Forsythe, 1987; Hazard & Ferro, 1991, Zhang & Sanderson, 1997). Whereas monophagous predators such as hoverflies must move to a different area or face starvation.

Although the percentage of carabids that feed on slugs and aphids is relatively constant in both the *Phacelia* treated and control plots, the increased numbers of carabids in the *Phacelia* treated plots relative to control plots mean there will be an overall increase in the numbers of carabids in *Phacelia* treated plots which are preying on both slugs and aphids. For example, in 1998 the mean percentage of carabids preying on aphids was approximately 40%, therefore, of the 80 beetles trapped in control plots only 30 would have preyed on aphids, while in *Phacelia* treated plots out of 619 beetles trapped, 247 would have been praying on aphids. Therefore, the overall rate of predation will be higher in *Phacelia* treated plots than in control plots and the carabids will make a much larger impact on pest populations in *Phacelia* flower extract treated plots

This suggests that the decline in aphid numbers in the *Phacelia* treated plots is due to an increase in the rate of predation bought about by increased numbers of predators attracted into the plot by the semiochemical.

8.5 Summary

- Barriers prevent the entry of carabids into field plots.
- *Phacelia* flower extract attracts increased numbers of carabids into field plots.
- In *Phacelia* non barriered plots aphid numbers are reduced.
- In *Phacelia* barriered plots, where carabid numbers are kept low, aphid numbers remain high
- Therefore *Phacelia* flower extract does not affect aphid behaviour, suggesting that aphid numbers are reduced by predation.
- ELISAs confirm that a high percentage of carabids trapped in the fields have been feeding on both slugs and aphids.
- Field, distance from field edge, and treatment have no effect on the percentage of carabids feeding on slugs and aphids.
- Time of the season does affect the percentage of carabids feeding on pest species, probably due to fluctuations in pest numbers.
- Although treatment does not affect the percentage of carabids feeding on aphids and slugs, *Phacelia* flower extract increases the number of carabids in the area, therefore increasing the level of predation in the plots relative to untreated plots.

Chapter 9

Summary and general discussion

Chapter 9: Summary and discussion

9.1 Discussion of techniques

The techniques utilised during this study were suitable in assessing the role of volatile semiochemicals in the attraction of invertebrate predators into crops in arable fields, and the subsequent effect on populations of economically important pest species.

The EAG system employed in this study was a standard set up similar to that used in other studies (Evans & Allen-Williams, 1989; Blackwell *et al.*, 1997), and proved to be an effective technique to determine the electrophysiological response of polyphagous carabids and monophagous hoverflies to range of volatile semiochemicals. EAG recordings were not made from *Coccinellidae* due to problems with background noise and inconsistency of response from the preparation. The use of EAGs indicated that the excised heads of male and female *P. melanarius* could detect cis-3-hexanol and *Phacelia* flower extract, and males could also detect (E)- β -farnesene. Male and female *N. brevicollis* exhibited antennal responses to cis-3-hexanol and (E)- β -farnesene, and females also responded to *Nepeta* extract. Male and female *E. balteatus* responded to cis-3-hexanol, *Phacelia* flower extract, (E)- β -farnesene and acetaldehyde, with females also able to detect *Nepeta* extract.

The four armed olfactometer design used in the study was useful in determining the behavioural response of the ground dwelling carabids to chemicals identified as inducing an electrophysiological response in the insects. The olfactometer offered

the insects a choice of up to four different odours which reduces the numbers of insects needed to satisfy statistical analysis (Vet *et al.*, 1983; Evans, 1991). Video recording equipment mounted over the olfactometer allowed the detection of a greater range of behaviours which may be missed by the human eye, and recording behaviours in the Observer software package made subsequent statistical analysis easier (Bakchine *et al.*, 1990). In the olfactometer, male and female *P. melanarius* spent significantly more time in the *Phacelia* flower extract odour field relative to the control fields, males also exhibited a similar response to (E)- β -farnesene. Only male *N. brevicollis* exhibited behavioural changes in the olfactometer in response to (E)- β -farnesene.

The major disadvantage of the four arm olfactometer system was that flight by insects under study was virtually impossible. Therefore the system was satisfactory to study the behavioural responses of insects who hunt by walking along the ground such as the carabids. However, it's unsuitable for insects such as syrphids that carry out long range orientation to wind borne odours during flight (Gilbert, 1993).

A wind tunnel was therefore employed to study the behavioural responses of hoverflies to sources of wind borne odour. The wind tunnel was based on the design of Hern (1997), however similar designs have been used in other studies (Tobin, 1981; Baker & Linn, 1984; Nottingham & Coaker, 1985). The wind tunnel was large enough to allow easy access and could accommodate large numbers of insects at once, for reducing the numbers of replicates needed for statistical significance (Hern, 1997). However, the perspex walls of the wind tunnel needed to be cleaned after every replicate to ensure that there were no traces of the test chemicals from the

previous trial. In the wind tunnel male and female *E. balteatus* exhibited a behavioural response to *Phacelia* flower extract, (E)- β -farnesene, and to acetaldehyde, females also responded to *Nepeta* extract. In most cases the response was indicative of positive upwind anemotaxis (Kennedy, 1977), with fewer individuals resting in the downwind section of the wind tunnel and significant increase in the number of hoverflies undergoing flight behaviour in all sections of the wind tunnel.

The equipment used for the field trials was simple to construct and set up but robust enough to survive long periods of time in the field. The techniques used to monitor both predator and pest populations such as pitfall trapping probably led to an a slight underestimation of total numbers of individuals in the populations (Schotzko & O'Keefe, 1989). However, they were chosen for the study as they are the least invasive methods of sampling causing minimum disruption to the system, and there was unlikely to be any differential attraction of insects to the trap which could affect the results (Broumas & Haniotakis, 1994). Therefore, the sampling methods involved provide an indication of the activity/ density of the insects involved (Schotzko & O'Keefe, 1989), which is important in this study as only active predators will reduce the populations of pest species. Artificial honeydew and L-tryptophan did not cause a significant increase in the number of carabid beetles in plots. However, *Phacelia* flower extract, *Nepeta* extract, (E)- β -farnesene, and Envirofeast™ all caused a significant increase in the number of active carabids present in treated plots. The treatments also led to an increase in the number of carabids at all distances throughout the field, whereas when attractive plants such as *Phacelia* were planted

around the field margin, there was only an increase in predator populations in the first 10m into the field (Holland *et al.*, 1994).

Barriers around the edge of field plots prevent the entry of carabids into the plot. *Phacelia* flower extract led to a significant increase in the number of carabids in non-barriered plots, and a corresponding fall in aphid numbers. However, when a barrier was placed around *Phacelia* treated plots carabid numbers within the plot remained low, and aphid populations stayed high. This suggests that *Phacelia* flower extract has no behavioural effect on the aphids themselves, and the reduction in aphid numbers is due to increased predation pressure.

ELISAs provide a valuable tool in quantifying the rates of predation in tritrophic systems by predators trapped in the field (Symondson & Liddel, 1996), and are useful in studying the link between predator and prey numbers in the field. However, the monoclonal antibodies employed were general to wide groups of pest species such as slugs and aphids. The antibodies were suitable for use in this study as a broad range of pests were monitored. For more specific studies on the control of particular pest species, specific monoclonal antibodies need to be used.

The use of ELISAs confirmed that a large percentage of the *P. melanarius* and *N. brevicollis* trapped had been feeding on both slugs and aphids in all years of the study. However, treatment, field, or distance from the field edge did not have a significant effect on the percentage of beetles feeding on these pest species. Only the time of sampling had any effect on the percentage of beetles containing prey protein in their gut contents, and this may be due to prey availability.

However, because the treatments did lead to a significant increase in the number of beetles in the plots, even though the percentage of beetles feeding on slug and aphids remains the same, the rate of predation in the treated plots will be increased relative to untreated plots.

9.2 Electrophysiological responses of predators

Electrophysiological studies confirm that the antenna of predators such as carabids and syrphids can detect and respond to a range of volatile chemicals arising from diverse sources including plants and insect prey. This supports the finding of both Wheeler (1989) and Hood-Henderson & Wellington (1982), who suggested that olfactory cues may play a significant role in resources finding by both carabids and syrphids.

The antenna of many groups of insects have receptor cells that are able to detect plant derived semiochemicals (Ramachandron & Norris, 1993). Herbivorous insects use the detection of plant derived semiochemicals as a first step to the location of food (Dickens *et al.*, 1993; Cosse *et al* 1995).

In predatory insects detection of plant derived semiochemicals would seem to be a waste of resources, as the predator feeds on other insects and not plants. However, in *E. balteatus* only the larval stages are predatory, the adults require both a nectar and pollen source to provide energy for flight and protein for oviposition (Gilbert, 1993). Therefore, plant derived volatiles, particularly ones arising from flowers such as *Phacelia*, may act as direct cues enabling *E. balteatus* to locate floral resources.

Adult *P. melanarius* and *N. brevicollis* are not known to feed on pollen or nectar and derive all of their resources from their prey (Luff, 1987). However, if they cannot locate cues arising directly from their prey species, then the ability to detect plant derived semiochemicals may prove to be a valuable asset, as it enables the beetles to locate habitat where prey species may be present.

Detection of insect derived semiochemicals by the antennae of other insects has shown to be extremely widespread (Shu *et al.*, 1998; Scholz *et al.*, 1998). This is the means that most insects govern interactions both within and between species (Roitberg & Isman, 1992). However, in predators detection of kairomones produced by other species of insects can be a precursor for the initiation of prey finding behaviour (Nordlund & Lewis, 1976). Detection of compounds such as (E)- β -farnesene, nepetalactol and nepetalactone may enable carabids to directly locate aphid prey. Adult syrphids are not predaceous (Gilbert, 1993), but prey derived semiochemicals may indicate a good site for oviposition, or a supply of other resources such as aphid honeydew a valuable secondary carbohydrate source (Frazer, 1972). Therefore detection of kairomones may be important in the location of non-prey resources.

The predator's antennae are able to detect changes in concentration of both plant and prey derived semiochemicals enabling the insect to make constant adjustments to its searching pattern as it moves nearer to or away from the site of highest concentration of semiochemicals (Warren *et al.*, 1996). This enables carabids and syrphids to carry out positive chemotaxis, as the highest concentration of semiochemicals will be

located near to the source, it may enable the predators to locate prey in the absence of other cues.

9.3 Behavioural responses of predators

E. balteatus, *P. melanarius* and *N. brevicollis* demonstrated positive orientation to semiochemicals in behavioural assays. This supports the findings of Wheeler (1989) and Kielty *et al.* (1995) who both found positive orientation in predators in laboratory studies. Semiochemicals which attracted predators in behavioural studies include those associated with prey species including (E)- β -farnesene, a major component of the aphid alarm pheromone (Dawson *et al.*, 1982), and acetaldehyde a constituent of aphid honeydews (Hood-Henderson & Wellington, 1982). They also include plant derived compounds such as *Phacelia* flower extract.

Volatile (E)- β -farnesene provides cues for direct location of aphid prey for polyphagous carabids, and therefore, when the carabids contact an odour plume containing this semiochemical they initiate a localised search pattern, which involves a decrease in walking speed and an increase in turning rate (Kielty *et al.*, 1995) which keeping the beetle in the odour field for longer periods and increasing its chance of locating prey (Kielty *et al.*, 1995). In the olfactometer this means that the beetle spends significantly longer in the treatment odour field than the control odour field.

(E)- β -farnesene also initiates a behavioural response in *E. balteatus* in the wind tunnel. However as *E. balteatus* forages for sources in flight (Gilbert, 1993) the behavioural response differs from that of the carabids. They undergo positive upwind

anemotaxis (Kennedy, 1977), flying upwind response to an odour plume. Therefore in the wind tunnel insects orientating to semiochemicals spend more time undergoing flight behaviour and generally move from the downwind section of the tunnel to the upwind section.

(E)- β -farnesene and acetaldehyde may provide cues for *E. balteatus* for the location of aphid honeydew (Gilbert, 1993). However they may also provide female *E. balteatus* with cues to locate suitable oviposition sites (Hood-Henderson & Wellington, 1982).

Phacelia flower extract induces behavioural responses in *E. balteatus* in the wind tunnel and in *P. melanarius* in the olfactometer. *Phacelia tanacetifolia* provides a pollen source for *E. balteatus* (Cowgill *et al.*, 1993a) and is readily visited by hoverflies when planted around the field margins (Hickman & Wratten, 1996). Attraction to *Phacelia* was thought to be due to visual cues (Holland *et al.*, 1994), however, the wind tunnel assays suggest that there may be a volatile component involved. *P. melanarius* is not known to feed on pollen and nectar (Luff, 1987), therefore volatile cues from *Phacelia* may act as habitat location cues, enabling the beetle to find habitat where prey may be present. Prey habitat location has been demonstrated in shore dwelling carabids, which use volatile substances produced by cyanobacteria in sand dunes to locate habitat where their prey of sand hoppers and other small crustaceans may be found (Evans, 1988).

9.4 Field responses

When placed in dispensers throughout the field system several semiochemicals lead to a significant increase in the number of carabids within an arable crop. These include (E)- β -farnesene, *Nepeta* extract and *Phacelia* flower extracts. This has previously been demonstrated with *Phacelia tanacetifolia* plants placed around the field margins (Cowgill *et al.*, 1993 a, b; Hickman & Wratten, 1993). However if semiochemical dispensers are employed instead of plants, then the farmer does not have to leave strips of land around the edges of the field and more area can be used to produce crops. Spraying food supplements onto crops has also been shown to lead to a significant increase in the numbers of beneficial insects in the crop (Ben Saad & Bishop, 1976). This was supported by the present study, but the increase was not as large as previously reported. However, food supplements may be used to maintain populations of beneficial insects attracted into the field by semiochemicals.

Plants attractive to predators when planted around the field margins only lead to an increase in the number of predators in the field system up to 10m from the field edge (Hickman & Wratten, 1996). Semiochemicals however, can be used to increase the number of predators throughout the field system so that effective control may be achieved by predators even in the middle of the field.

Treated field plots with increased numbers of carabids exhibited a corresponding fall in aphid populations relative to the control untreated plots. The reduction in aphid populations may be due to two factors; direct behavioural effect of the semiochemicals on the aphids themselves (Dawson *et al.*, 1990), or increased predation pressures from the greater number of predators in the plot (Holland &

Thomas, 1997). However aphids become habituated to pheromones after prolonged exposure (Elagamy & Haynes, 1992), reducing the chances that behavioural effects of the semiochemicals could be responsible for long term reduction in aphid populations.

Barriers placed around field plots reduced colonisation of the plots by carabids. In treated plots without barriers there were increased carabid numbers and a reduction in aphid populations relative to non treated controls. However, if treated plots are surrounded by barriers there is no significant increase in carabid numbers and aphid populations remain roughly equal to those in control plots. This suggests that predators are responsible for the decline in aphid numbers which supports the findings of Holland & Thomas (1997).

ELISA assays on the gut contents of captured carabids indicate that the carabids regularly feed on both slugs and aphids, supporting the findings of Symondson *et al.* (1996).

Although the majority of captured beetles' guts contained both slug and aphid proteins, not all of them did supporting the findings of Symondson & Liddel (1993). Both treatment and field factors did not have a significant effect on the percentage of beetles that were feeding on these pest species. This may be due to prey preference factors in the predators (Bilde & Toft, 1994; 1997), with only a certain percentage of the beetles exhibiting preference for these prey items. The only factor affecting the percentage of predators feeding on aphids and slugs was sampling date. This was probably because aphid and slug numbers are not constant and change throughout the field season, and if a prey species has a low abundance then predators will have

less success in finding it, therefore fewer individuals of that species will be consumed.

However, even though the semiochemical treatments do not increase the proportion of beetles feeding on slugs and aphids in the field plots, they do lead to a significant increase in carabid populations. Therefore the rates of predation in treated plots will be increased relative to control plots where carabid numbers remain low, for example if 80% of 100 beetles in treated plots feed on slugs and aphids this will lead to a 10 fold increase in the rates of predation relative to the 80% of the 10 beetles in the control plots feeding on slugs and aphids.

One strategy to exploit the attraction of predators to these semiochemicals would be to place semiochemical dispensers in the field at the beginning of the field season after the crops have been sown. The dispensers would release their semiochemicals over the entire field season, attracting in different predators over the entire season. Early emerging carabids such as *N. brevicollis* would be attracted into the fields at the beginning of the season and prey on invertebrate pests before the populations have a chance of become established thus preventing population increase. When *N. brevicollis* enters its summer diapause other species such as *P. melanarius* become much more active and take over the role of biological control agent. The semiochemicals can also be used to maintain populations of polyphagous predators in the field even when pest populations are low, as the semiochemical cues from the dispensers which indicate to the predator that prey may still be in the area, and polyphagous predators can maintain themselves on alternative prey species such as collembola and other invertebrates (Luff, 1987). The carabids may leave the area

eventually if habituation to the chemicals eventually occurs, or if the hunting predator continually fails to find prey. However, the beetles that leave may be replaced by continuous re-colonisation of the field by beetles from outside of the field system attracted in by the semiochemicals. If local populations of other predators are high the chemicals may also be used to attract these predators into the field, enhancing natural control even more.

9.5 Potential for use in IPM schemes

If used in conjunction with integrated pest management systems, semiochemicals may lead to a reduction in pesticide application. However, if semiochemical treatments are used in conjunction with chemical insecticides then the timing of application of the pesticide is a very important factor that must be considered. Chemical insecticides not only reduce populations of pests in crops, they also have a detrimental effect on beneficial arthropods present in the field system (Hawthorne & Hassal, 1994). In integrated cotton production in Australia these problems are overcome by spraying with chemical insecticides very early in the season when pest populations are becoming established, but before predatory insects enter the field (Fitt, 1994). For the rest of the growing season no other chemicals are used and natural control relied on. The predators naturally present in the field are able to control the reduced pest populations. (Fitt, 1994). Semiochemicals could be used to enhance the system by attracting in increased numbers of predators at the appropriate time, reducing the initial amount of pesticide that would have to be applied to achieve effective control.

9.6 Future work

Although the volatile components of *Phacelia* flower extract have been identified the active components are not known. Therefore the chemicals which make up the *Phacelia* flower volatiles should be tested individually in electrophysiological and behavioural assays to determine which of the volatiles in the *Phacelia* mixture are the biologically active components causing greatest attraction to the predators. It may then be possible to chemically synthesise these volatiles in the laboratory in greater amounts and higher purity than that produced by the plants. These may then be evaluated in the field to determine whether they cause larger increases in the predator populations.

Field trials using the identified semiochemicals should also be carried in other areas of the country where populations of other predator groups such as the *Syrphidae*, *Staphylinidae*, *Coccinellidae* and *Chrysopidae* are larger than the populations of South East Scotland, to determine the effects of these chemicals on these predators in the field.

Further work could be carried out on the method of application of the semiochemicals. Single chemicals such as (E)- β -farnesene, or the active components of the plant extracts could be assessed for their suitability to be incorporated into slow release plastic strips. Research would then need to be carried out to determine the optimum number and spacing of the slow release dispensers in the field to have greatest effect on predator numbers. Slow release systems have been previously used in the past to release oviposition repellents for *Phorbia brassica* Buche to protect cauliflower plants. The repellents were entrapped in slow release granules, which

protected the plants for up to 5 weeks (Denouden *et al.*, 1993). Slow release dispensers would also make the process less labour intensive and therefore more attractive to farmers and landowners.

Field trials should also be carried out to determine if the semiochemicals could be used in conjunction with food supplements such as Envirofeast™ in the same field to determine if this would have a synergistic effect on populations of beneficial insects. Yield studies could also be carried out on the crop to determine if the semiochemicals lead to an increase in yield and therefore greater profits for the farmer.

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Appendices

Appendix 1 EAG P values

Table 10.1 EAG P values for *P. melanarius* (n=6).

Semiochemical	P value	
Acetaldehyde	male and female	0.937
Artificial honeydew	male and female	0.813
β -caryophellene	male and female	0.97
β -hydroxybutyric acid	male and female	0.99
cis-3-hexanol	male and female	5.49×10^{-10}
(E)- β -farnesene	male 0.0014	female 0.058
L-tryptophan	male and female	0.071
<i>Nepeta</i> extract	male and female	0.107
<i>Phacelia</i> flower extract	male and female	0.022
<i>Phacelia</i> leaf extract	male and female	0.782

Table 10.2 EAG P values for *N. brevicollis* (n=6).

Semiochemical	P value	
Acetaldehyde	male and female	0.3
Artificial honeydew	male and female	0.131
β -caryophellene	male and female	0.41
β -hydroxybutyric acid	male and female	0.51
cis-3-hexanol	male and female	5.12×10^{-10}
(E)- β -farnesene	male and female	0.231
L-tryptophan	male and female	0.971
<i>Nepeta</i> extract	male 0.44	female 0.0098
<i>Phacelia</i> flower extract	male and female	0.078
<i>Phacelia</i> leaf extract	male and female	0.92

Table 10.3 EAG P values for *E. balteatus* (n=6).

Semiochemical	P value	
Acetaldehyde	male 0.3	female 0.026
Artificial honeydew	male and female	0.541
β -caryophellene	male and female	0.543
β -hydroxybutyric acid	male and female	0.935
cis-3-hexanol	male and female	5.12×10^{-10}
(E)- β -farnesene	male and female	3.5×10^{-7}
L-tryptophan	male and female	0.99
<i>Nepeta</i> extract	male 0.57	female 0.0098
<i>Phacelia</i> flower extract	male and female	0.022
<i>Phacelia</i> leaf extract	male and female	0.99

Appendix 2 P values from behavioural assays

Table 10.4 P values for *P. melanarius* in the four armed olfactometer (n=20)

Semiochemical	Males P value	Females P value
Methanol (control)	0.31	0.95
(E)- β -farnesene	0.013	0.29
<i>Nepeta</i> extract	0.65	0.99
<i>Phacelia</i> flower extract	0.001	0.0098

Table 10.5 P values for *P. melanarius* in the four armed olfactometer (n=20)

Semiochemical	Males P value	Females P value
Methanol (control)	0.46	0.70
(E)- β -farnesene	0.001	0.38
<i>Nepeta</i> extract	0.85	0.82
<i>Phacelia</i> flower extract	0.58	0.77

Table 10.6 P values for *E. balteatus* in the wind tunnel (n=3)

Semiochemical	Males P value	Females p value
Acetaldehyde	8.87×10^{-10}	3.41×10^{-5}
(E)- β -farnesene	0.0056	4.76×10^{-5}
<i>Nepeta</i> extract	0.244	10.39×10^{-10}
<i>Phacelia</i> flower extract	8.1×10^{-4}	4.81×10^{-10}

Appendix 3 P values from 1996 field studies

Table 10.7 P values for carabids trapped per week in the 1996 field season (n=3).

Semiochemical	P value
Artificial Honeydew	0.044
(E)- β -farnesene ground	0.009
(E)- β -farnesene canopy	8.02×10^{-8}
Envirofeast TM	0.0028
L-tryptophan	1.98×10^{-5}
<i>Nepeta</i> extract ground	10.84×10^{-7}
<i>Nepeta</i> extract canopy	7.35×10^{-5}
<i>Phacelia</i> flower extract ground	4.94×10^{-8}
<i>Phacelia</i> flower extract canopy	6.91×10^{-6}

Table 10.8 P values for carabids trapped per field in the 1996 field season (n=3).

Semiochemical	Winter wheat	Spring barley
Artificial Honeydew	0.99	-
(E)- β -farnesene ground	0.0834	0.039
(E)- β -farnesene canopy	4.24×10^{-4}	0.39
Envirofeast TM	-	0.086
L-tryptophan	0.184	-
<i>Nepeta</i> extract ground	2.96×10^{-5}	0.012
<i>Nepeta</i> extract canopy	0.02	0.97
<i>Phacelia</i> flower extract ground	3.44^{-8}	0.67
<i>Phacelia</i> flower extract canopy	0.0031	0.016

Table 10.9 P values for aphids trapped per field in the 1996 field season (n=3).

Semiochemical	Winter wheat	Spring barley
Artificial Honeydew	10.99×10^{-4}	-
(E)- β -farnesene ground	5.52×10^{-14}	0.012
(E)- β -farnesene canopy	1.18×10^{-14}	0.0069
Envirofeast TM	-	0.028
L-tryptophan	7.43×10^{-11}	-
<i>Nepeta</i> extract ground	8.43×10^{-15}	0.012
<i>Nepeta</i> extract canopy	4.78×10^{-15}	0.342
<i>Phacelia</i> flower extract ground	10.06×10^{-16}	0.0017
<i>Phacelia</i> flower extract canopy	10.86×10^{-10}	0.228

Appendix 4. Total numbers of Coccinellids and Syrphids trapped in the 1996 and 1997 field seasons.**Table 10.10 Total number of Coccinellids and Syrphids trapped in 1996 and 1997**

Predator	1996	1997
<i>Coccinellidae</i>	23	27
<i>Syrphidae</i>	12	7

Appendix 5 P values from field studies in 1997.**Table 10.11 P values for carabids trapped in 1997 (n=3)**

Factor	P value
field	0.98
distance	0.048

Table 10.12 P values for carabids trapped in the treated plots in 1997 (n=3)

Treatment and distance	P value
(E)- β -farnesene 0-10m	0.988
(E)- β -farnesene 45-55m	0.0021
(E)- β -farnesene 90-100m	0.0016
Envirofeast TM 0-10m	0.98
Envirofeast TM 45-55m	0.23
Envirofeast TM 90-100m	0.21
<i>Nepeta</i> extract 0-10m	0.162
<i>Nepeta</i> extract 45-55m	0.0067
<i>Nepeta</i> extract 90-100m	0.002
<i>Phacelia</i> flower extract 0-10m	0.411
<i>Phacelia</i> flower extract 45-55m	0.002
<i>Phacelia</i> flower extract 90-100m	4.01×10^{-10}

Table 10.13 P values for aphids trapped in 1997 (n=3)

Factor	P value
field	0.011
distance	0.56

Table 10.14 **P values for aphids trapped in the treated plots in 1997 (n=3)**

Treatment and distance		P value
(E)- β -farnesene	0-10m	3.38×10^{-5}
(E)- β -farnesene	45-55m	0.0016
(E)- β -farnesene	90-100m	7.96×10^{-9}
Envirofeast TM	0-10m	3.0×10^{-5}
Envirofeast TM	45-55m	0.055
Envirofeast TM	90-100m	0.12
<i>Nepeta</i> extract	0-10m	0.0034
<i>Nepeta</i> extract	45-55m	0.0067
<i>Nepeta</i> extract	90-100m	0.002
<i>Phacelia</i> flower extract	0-10m	1.62×10^{-5}
<i>Phacelia</i> flower extract	45-55m	0.002
<i>Phacelia</i> flower extract	90-100m	6.76×10^{-5}

Appendix 6 P values from field studies in 1998**Table 10.15 P values for carabids trapped in the 1998 field season (n=3).**

Field plots	P value
<i>Phacelia</i> and control	2.06×10^{-7}
<i>Phacelia</i> barriered and control	0.36
control barriered and non-barriered	0.215
<i>Phacelia</i> barriered and non-barriered	3.89×10^{-7}

Table 10.16 P values for aphids trapped in the 1998 field season (n=3).

Field plots	P value
<i>Phacelia</i> and control	4.16×10^{-14}
<i>Phacelia</i> barriered and control	0.41
control barriered and non-barriered	0.41
<i>Phacelia</i> barriered and non-barriered	6.19×10^{-20}

Appendix 7 P values for ELISA's**Table 10.17 P values for aphid protein from carabids trapped in 1996.**

Factor	<i>P. melanarius</i> P values	<i>N. brevicollis</i> P values
field	0.7	0.5
treatment	0.1	0.95
date	0.001	0.01

Table 10.18 P values for slug protein from carabids trapped in 1996.

Factor	<i>P. melanarius</i> P values	<i>N. brevicollis</i> P values
field	0.8	0.9
treatment	0.7	0.11
date	0.01	0.001

Table 10.19 P values for aphid protein from carabids trapped in 1997.

Factor	<i>P. melanarius</i> P values	<i>N. brevicollis</i> P values
field	0.7	0.9
distance	0.5	0.7
treatment	0.9	0.5
date	0.001	0.001

Table 10.20 P values for slug protein from carabids trapped in 1997.

factor	<i>P. melanarius</i> P values	<i>N. brevicollis</i> P values
field	0.99	0.7
distance	0.99	0.3
treatment	0.99	0.11
date	0.001	0.001

Table 10.21 P values for aphid protein from carabids trapped in 1996.

Factor	<i>P. melanarius</i> P values	<i>N. brevicollis</i> P values
field	0.9	0.5
treatment	0.3	0.1
date	0.001	0.001

Table 10.22 P values for slug protein from carabids trapped in 1996.

Factor	<i>P. melanarius</i> P values	<i>N. brevicollis</i> P values
field	0.98	0.2
treatment	0.7	0.1
date	0.001	0.01

Publications arising from this study

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Manipulating the behaviour of beneficial insects in cereal crops to enhance control of aphids

D.L. Kirkland, K.A. Evans & T. Lola-Luz
SAC, West Mains Road, Edinburgh EH9 3JG, UK

ABSTRACT

The release of a synthesised analogue of the aphid alarm pheromone, (E)- β -farnesene from dispensers sited in winter wheat crops significantly affected the activity of carabid beetles, aphid numbers on plants and levels of aphid parasitism.

The potential uses of this approach for the management of aphids is discussed.

INTRODUCTION

The use of semiochemicals as part of an integrated approach for the control of aphid pests has been suggested by Leszczynski *et al.* (1995) and Losel *et al.* (1996).

Predators and parasitoids have been shown to use prey-derived semiochemicals as long range orientation cues (Bowers & Borden, 1992; Grasswitz & Paine, 1992), and (E)- β -farnesene, the aphid alarm pheromone, has been shown to elicit long range orientation in polyphagous predators (Kielty *et al.*, 1996).

Many potential semiochemicals of prey and plant origin have been screened in the laboratory and field for their potential use as attractants for predatory and parasitic insects, the full results of which will be published elsewhere. This paper illustrates some of the results obtained with manipulating the activity of carabid beetles within crops, and of levels of aphid parasitism by hymenopteran parasitoids, by an analogue of the aphid alarm pheromone, (E)- β -farnesene.

MATERIALS AND METHODS

(E)- β -farnesene (EBF) was prepared in the laboratory in a hydration reaction from nerolidol according to the method of Dawson *et al.* (1982). NMR confirmed the EBF as being 70% pure, the remainder being unreacted nerolidol and trace amounts of other β -farnesene isomers. The EBF was stored at 4°C.

Field experiments were carried out from the end of May, 1996 and May 1997 for seven weeks in a crop of winter wheat (cv. Riband - 1996; c.v. Avalon - 1997) at SAC, Edinburgh.

In 1996, the crop was split into 50m² plots, at the centre of which was a 10m² experimental plot. Various treatments were used but only the results for the following are presented: Control plots (95% methanol) (n = 3); 1% EBF dispensed at crop canopy height (n = 3); 1% EBF dispensed at ground height (n = 3).

Wick dispensers contained 1% EBF in methanol and were placed at the centre of the 10m² experimental plots, and were replenished weekly. Once a week from the beginning of May, five pitfall traps sited at random within the experimental plots were uncovered overnight and the contents collected and identified the following day.

Ten wheat plants were taken at random once a week from the experimental plots and the numbers of aphids present determined.

In May 1997, wick dispensers containing 7% EBF in methanol were placed at crop canopy height 10m from the crop edge and every 20m until 70m from the edge of the crop (n = 4) along a transect from the crop edge. Control plots (n = 2) consisted of dispensers of 95% methanol alone 10m and 30m from the crop edge.

Ten plants, 1m apart, were labelled along a diagonal across the position of the dispenser. At weekly intervals the numbers of aphids were recorded from each marked plant. Aphid 'mummies' were recorded as being aphids parasitised by hymenopteran parasitoids.

RESULTS

1996

The activity of carabid beetles measured by the mean numbers caught in pitfall traps is shown in Fig. 1. With 1% EBF released at canopy level there was a significant increase in beetle numbers compared to the untreated ($P < 0.05$, analysis of variance). EBF released at ground level did not demonstrate a significant increase in beetle numbers.

Aphid (*Sitobion avenae*) numbers on plants from plots with no EBF release had significantly greater numbers of aphids compared to both EBF treatments (Fig. 2, $P < 0.05$).

1997

Analysis of variance of the data indicated no effect of distance from the dispenser on the numbers of aphids or parasitised aphids (*S. avenae*) on individual wheat plants, and there were no differences between each replicate for either treatment. Consequently the data from each replicate were combined to obtain the mean number of aphids/10 plants/replicate for the EBF and control treatments on each sampling date.

There were significantly fewer aphids on plants around the EBF dispensers compared to the control dispensers (Fig. 3, $P < 0.001$). Aphid counts remained relatively high

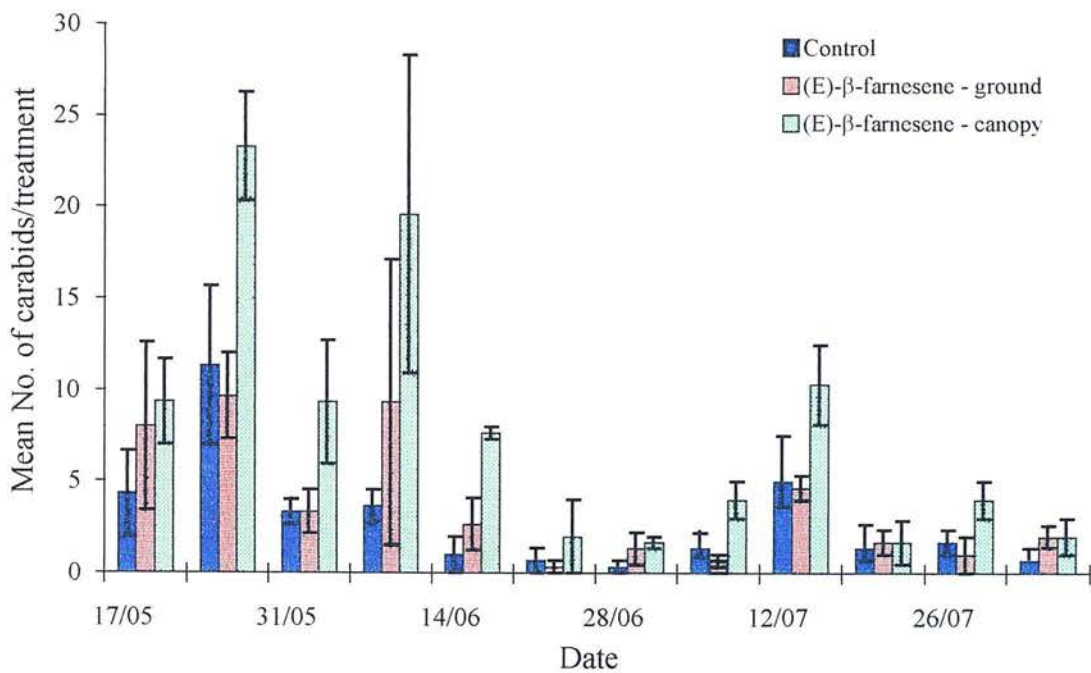


Figure 1. Mean No. (\pm SE) of carabid beetles/treatment caught overnight in 5 pitfall traps within the experimental area of winter wheat in 1996.

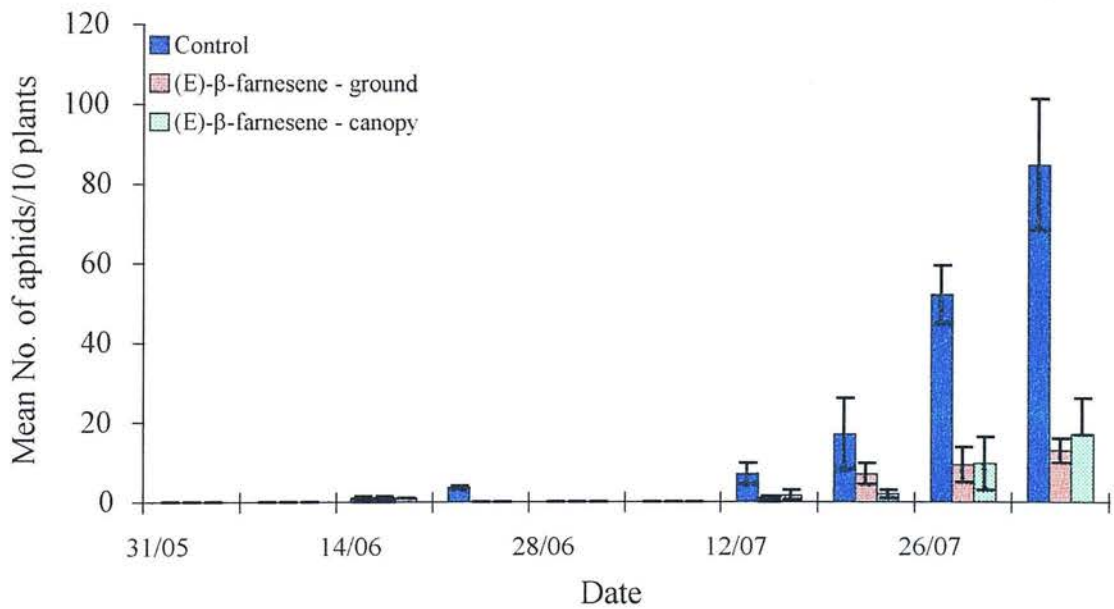


Figure 2. Mean No. (\pm SE) of aphids/10 plants/replicate within the experimental area of winter wheat in 1996.

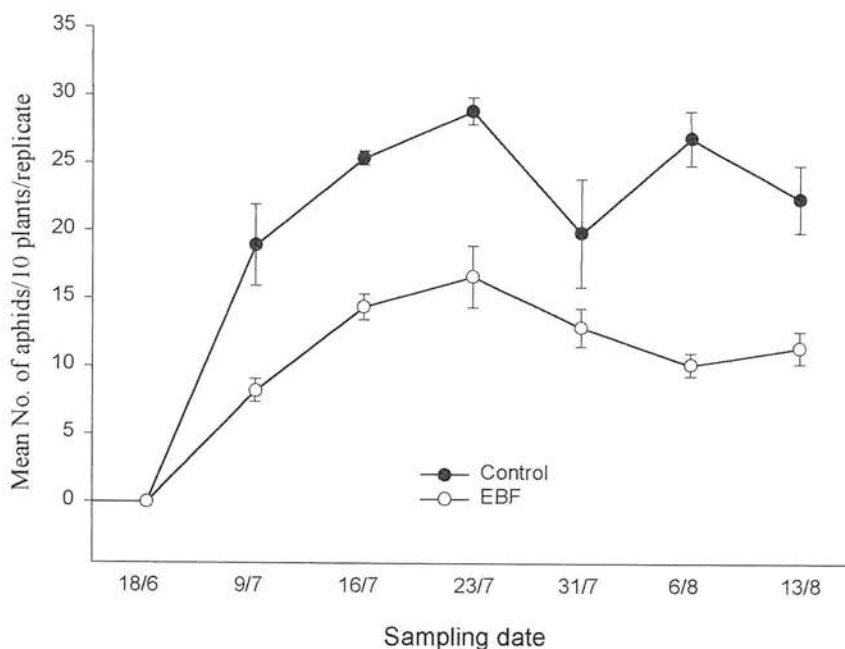


Fig. 3. Mean No. of *S. avenae* (\pm SE) from 10 plants in areas of a winter wheat crop with control dispensers (—●—, $n = 2$) and EBF dispensers (—○—, $n = 4$).

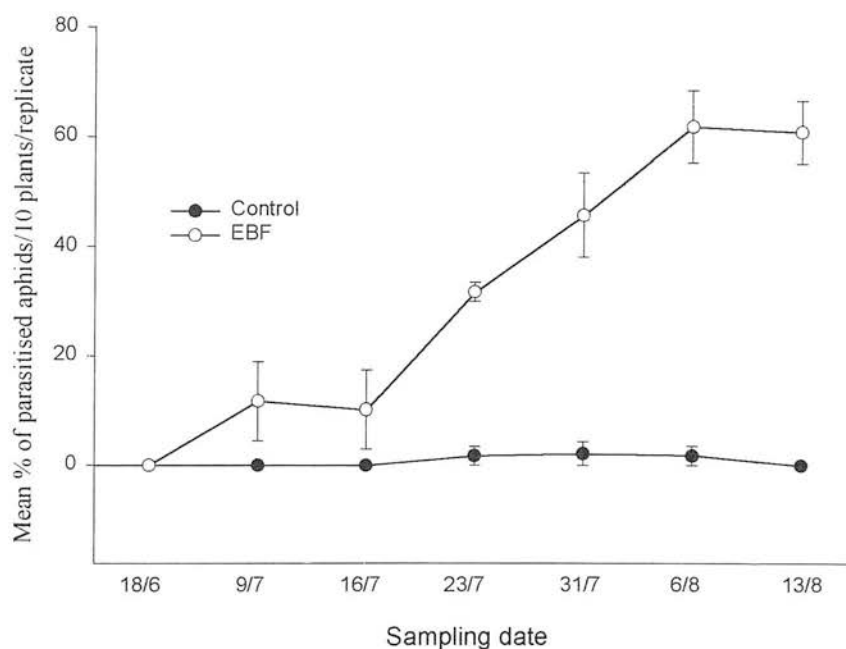


Fig. 4. Mean % of parasitised aphids (\pm SE) in areas of a winter wheat crop with control dispensers (—●—, $n = 2$) and EBF dispensers (—○—, $n = 4$).

(> 20 per replicate) in the control treatment after 4 weeks, whereas the level of aphid infestation was consistently 40-50% less in the EBF treatment (Fig. 3).

There was a significant difference in the level of aphid parasitism by parasitoids between the two treatments (Fig. 4, $P < 0.001$). Over 60% parasitism was recorded in the EBF treatments, whereas in the control treatments, parasitism peaked at 2.5% on week 6 (Fig. 4).

DISCUSSION

Synthesised aphid alarm pheromone, EBF, when released from dispensers in a winter wheat crop significantly alters the activity of an assemblage of carabid beetles, and increases the level of aphid parasitism by hymenopteran parasitoids. There is also a corresponding reduction in the level of aphid infestation.

Semiochemical-mediated habitat location has been reported in several carabid beetle species by Evans (1988, 1994), and adults of the carabid beetle *Pterostichus melanarius*, which was the most common beetle found in this study, orient to olfactory cues arising directly from prey species such as crickets and blowfly larvae (Wheater, 1989) and from analogues of the pheromones of prey species such as (E)- β -farnesene (Kielty *et al.*, 1995; Kirkland & Evans unpubl.).

Polyphagous carabids have an advantage in integrated pest management programs, in that they can survive in an area when there are no pest species present by feeding on alternative prey such as Collembola (Wheater, 1989). Therefore inducing carabid beetle movement into cereal fields using semiochemicals early in the season before pest populations become established will not lead to starvation or death of the beetles. Consequently beetles will be present within the crop when pests such as aphids arrive in the spring/early summer, enabling the beetles to reduce the pest population when numbers are low preventing a build up to damaging levels later in the season.

Aphid counts in the vicinity of EBF dispensers were significantly lower than those around control dispensers. This may well be due to the natural effect of the EBF on the behaviour of aphids by disturbing settling behaviour and/or leading to aphids dispersing from wheat plants as reported by Phelan *et al.* (1976); Nault & Montgomery (1979) and Wohlers (1981). Additionally reduction in aphid numbers by EBF may well be due to predation by coccinellids and syrphids, but few of these were noted in the crop until aphid levels peaked, and none were found on the plants sampled. Numbers of aphids climbing back onto plants after dropping off in response to EBF may be reduced by injury caused by the relatively long drop to the soil surface, and by predation by ground-dwelling generalist predators such as carabid beetles which are attracted to EBF (Kielty *et al.*, 1996; Kirkland & Evans, unpubl.) and which are more active in areas where EBF is being released.

There was a significant level of parasitism of aphids on plants in the vicinity of the EBF dispensers. Up to 60% parasitism was recorded in the form of aphid 'mummies' being present. Parasitism in areas around the methanol dispensers did not exceed 2.5%. Whilst evidence exists for attraction of aphid parasitoids to the aphid sex pheromone (+)-(4aS, 7S, 7aR)-nepetalactone (Hardie *et al.*, 1994) there are no reports of attraction to EBF. By releasing EBF via field dispensers, movement of the

parasitoids into the crop is enhanced, leading to greater levels of parasitism. Plants 70m from the crop edge did not show any differences in their level of aphid infestation or parasitism from plants closer to the edge of the field.

By using semiochemicals such as EBF to enhance the suite of predatory and parasitic insects within a crop, pests may be prevented from reaching levels economically damaging to the crop without recourse to the use of pesticides.

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AROMATHERAPY FOR CROPS: ENHANCING NATURAL CONTROL OF
GRAIN APHIDS IN CEREALS

D.L. Kirkland, T. Lola-Luz and K.A. Evans

Crop Health Department, SAC Edinburgh,
West Mains Road, EDINBURGH EH9 3JG

Summary: Dispensers of the semiochemicals (E)- β -farnesene and *Phacelia tanacetifolia* flower extract when placed within a crop of winter wheat significantly increased the activity of carabid beetles as measured by pitfall trap catches, increased aphid parasitism, and reduced aphid numbers on plants compared to untreated areas of the crop. The reasons for this and the potential use of these semiochemicals in integrated pest management are discussed.

INTRODUCTION

It is well established that many insect pests of arable crops use the smell of the crop to aid them in its location, however the response of beneficial insects to plant, or prey odour (semiochemicals) has not been studied in much detail, especially under field conditions. Many potential semiochemicals of prey and plant origin have been screened in our laboratory and in the field for their potential use as attractants for predatory and parasitic insects, the full results of which will be published elsewhere. This paper illustrates some of the results obtained regarding manipulating the activity of carabid beetles within crops, and levels of aphid parasitism by hymenopteran parasitoids by an analogue of the aphid alarm pheromone, (E)- β -farnesene (EBF), and an extract of flowers of *Phacelia tanacetifolia*. These semiochemicals were chosen as EBF has been shown to elicit long range orientation in polyphagous predators (Kielty *et al.*, 1996), and numbers of the aphidophagous hoverfly, *Episyrphus balteatus* were increased significantly by planting strips of *Phacelia* around field edges (Hickman & Wratten, 1996).

MATERIALS AND METHODS

EBF was prepared in the laboratory in a hydration reaction from nerolidol according to the method of Dawson *et al.* (1982). NMR confirmed the EBF as being 70% pure, the remainder being unreacted nerolidol and trace amounts of other β -farnesene isomers. The EBF was stored at 4°C until used. *P. tanacetifolia* was grown from seed (Chiltern Seeds, Cumbria, UK) in John Innes Irish moss peat in a glasshouse at

18 ± 4°C with a 16:8 hour light: dark regime. *Phacelia* flowers were harvested and mixed with methanol in a ratio of 1:10 (plant material: methanol), mixed in a blender and filtered under reduced pressure to obtain the *Phacelia* extract, which was stored at 4°C.

Field experiments were carried out from the end of May, 1996 and May 1997 for seven weeks in two crops of winter wheat (cv. Riband - 1996, Boghall Farm, East Lothian; cv. Avalon - 1997, Braid Hills Farm, Midlothian).

In 1996, the crop was split into 50m² plots, at the centre of which was a 10m² experimental plot. Various treatments were used but only the results for the following are presented: Control plots (95% methanol) (n = 3); 1% EBF dispensed at crop canopy height (n = 3); 1% EBF (in 95% methanol) dispensed at ground height (n = 3); 1% *Phacelia* extract (in 95% methanol) at crop canopy height (n = 3) and 1% *Phacelia* extract at ground height (n = 3). The wick dispensers containing the treatments were placed at the centre of the 10m² experimental plots, and were replenished weekly. Once a week from the beginning of May, five pitfall traps sited at random within the experimental plots were uncovered overnight and the contents collected and identified the following day. Ten wheat plants were taken at random once a week from the experimental plots and the numbers of grain aphids (*Sitobion avenae*) present determined.

In May 1997, wick dispensers containing 7% EBF in methanol (n = 4) or 1% *Phacelia* extract (n = 3) were placed at crop canopy height 10m from the crop edge and every 20m along a transect from the crop edge. Control plots (n = 2) consisted of dispensers of 95% methanol alone 10m and 30m from the crop edge. Ten plants, 1m apart, were labelled along a diagonal across the position of the dispenser. At weekly intervals the numbers of aphids were recorded from each marked plant. Aphid 'mummies' were recorded as being aphids parasitised by hymenopteran parasitoids.

RESULTS

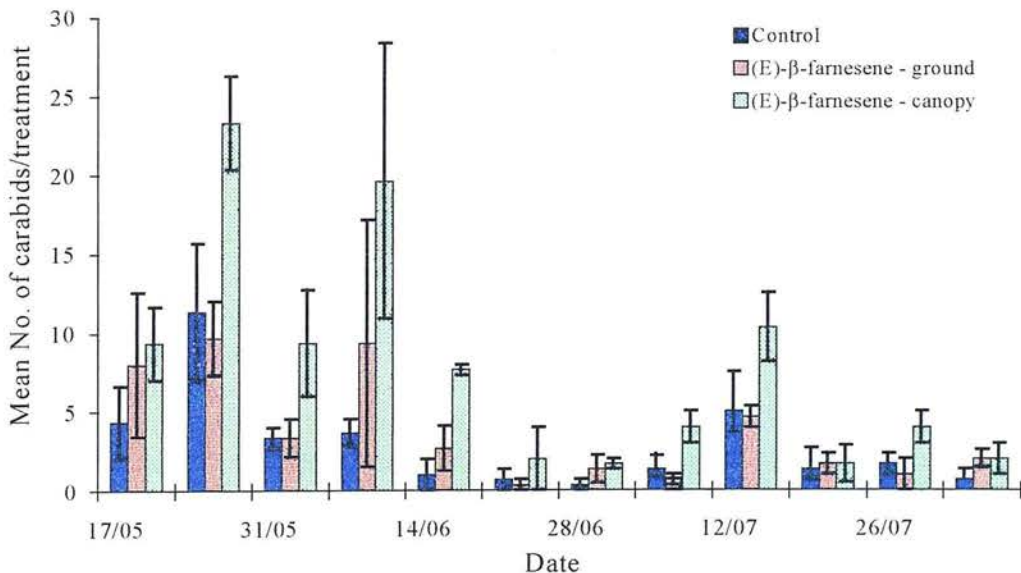


Fig1. Mean No. (± SE) of carabid beetles/treatment caught overnight in 5 pitfall traps within the EBF experimental area of winter wheat in.

The activity of carabid beetles measured by the mean numbers caught in pitfall traps is shown in Figs. 1 and 2. With 1% EBF released at canopy level there was a significant increase in beetle numbers compared to the untreated (Fig. 1, $P < 0.05$, analysis of variance). EBF released at ground level did not demonstrate a significant increase in beetle numbers.

Phacelia extract released at ground and canopy level led to a significant increase in beetle numbers compared to the untreated (Fig. 2, $P < 0.05$, analysis of variance).

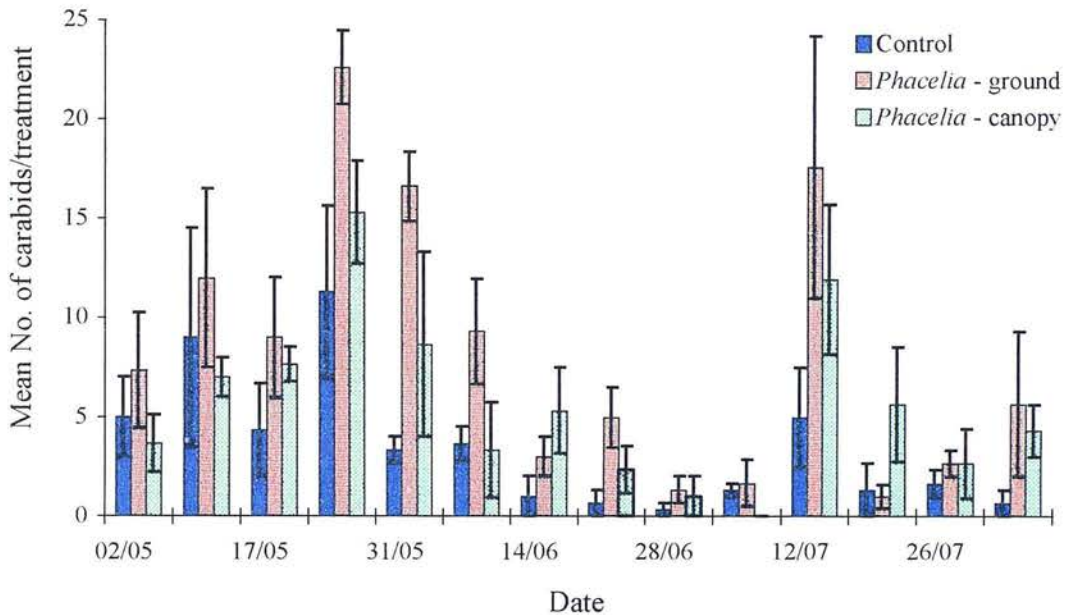


Fig. 2. Mean No. (\pm SE) of carabid beetles/treatment caught overnight in 5 pitfall traps within the *Phacelia* experimental area of winter wheat in 1996.

Aphid numbers

Grain aphid (*Sitobion avenae*) numbers on plants from plots with no EBF or *Phacelia* extract release were significantly greater compared to both EBF and *Phacelia* treatments (Figs. 3 and 4, $P < 0.05$).

Aphid parasitism

Analysis of variance of the data indicated no effect of distance from the dispenser on the numbers of aphids (*S. avenae*) or parasitised aphids on individual wheat plants, and there were no differences between each replicate for each treatment. Consequently the data from each replicate were pooled to obtain the mean number of aphids/10 plants/replicate for the EBF, *Phacelia* and control treatments on each sampling date.

There were significantly fewer aphids on plants around the EBF and *Phacelia* dispensers compared to the control dispensers (Fig. 5, $P < 0.001$).

There was a significant difference in the level of aphid parasitism by parasitoids between the two treatments (Fig. 6, $P < 0.001$). Over 60% parasitism was recorded in the EBF treatment, and over 70% in the *Phacelia* treatment, whereas in the control treatment, parasitism peaked at 2.5% (Fig. 6).

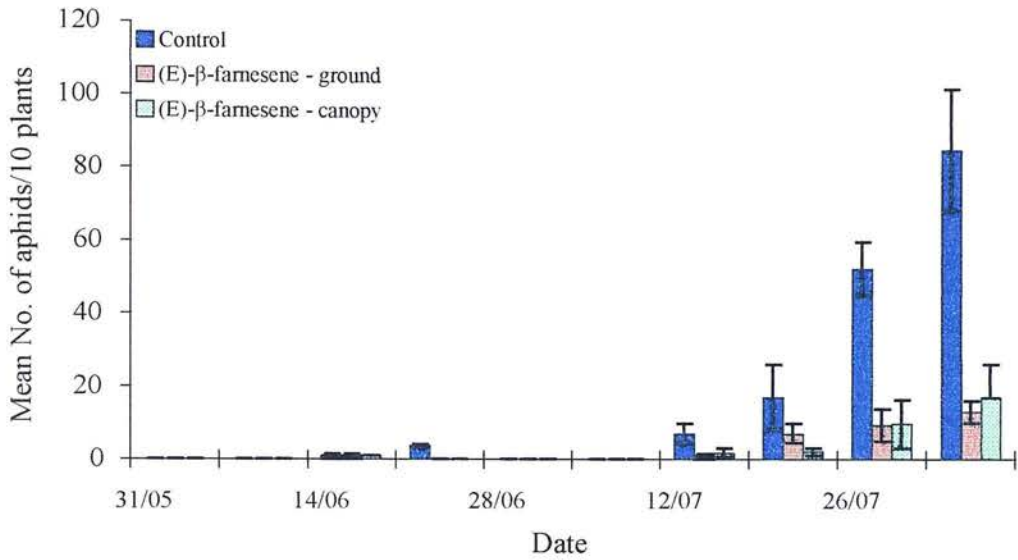


Fig. 3. Mean No. (\pm SE) of aphids/10 plants/replicate within the EBF experimental area of winter wheat in 1996.

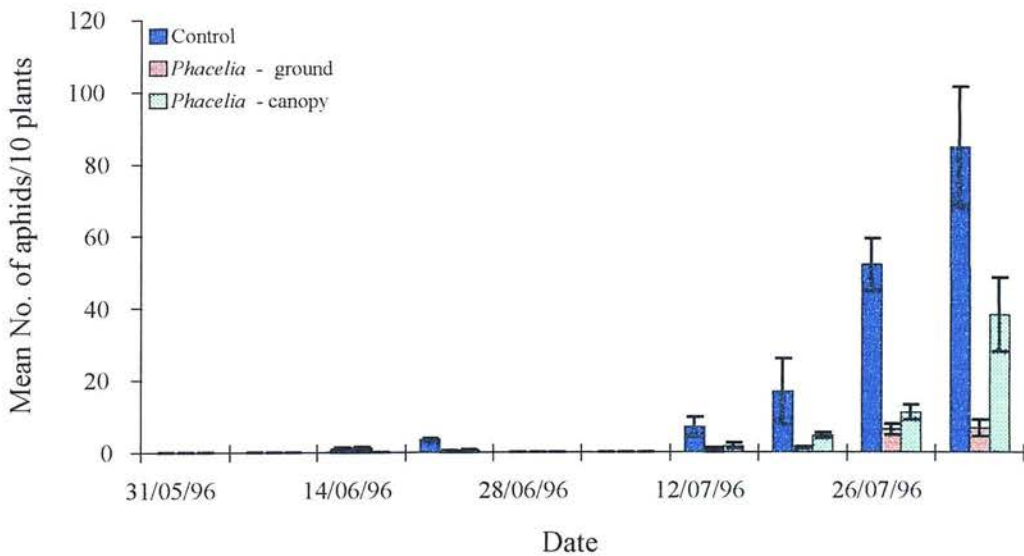


Fig. 4. Mean No. (\pm SE) of aphids/10 plants/replicate within the *Phacelia* experimental area of winter wheat in 1996.

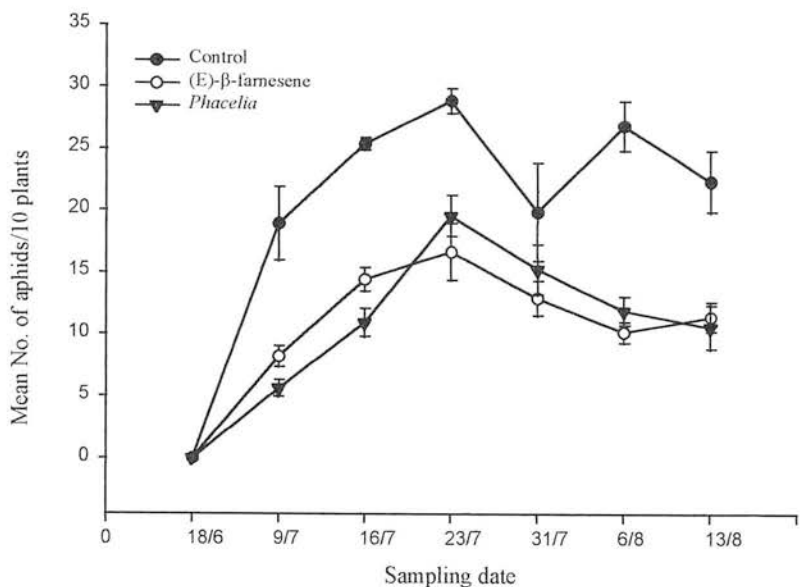


Fig. 5. Mean No. of aphids (\pm SE) in areas of a winter wheat crop with control dispensers (—●—, $n = 2$), (E)-β-farnesene dispensers (—○—, $n = 4$), *Phacelia* dispensers (—▼—, $n = 3$) in 1997.

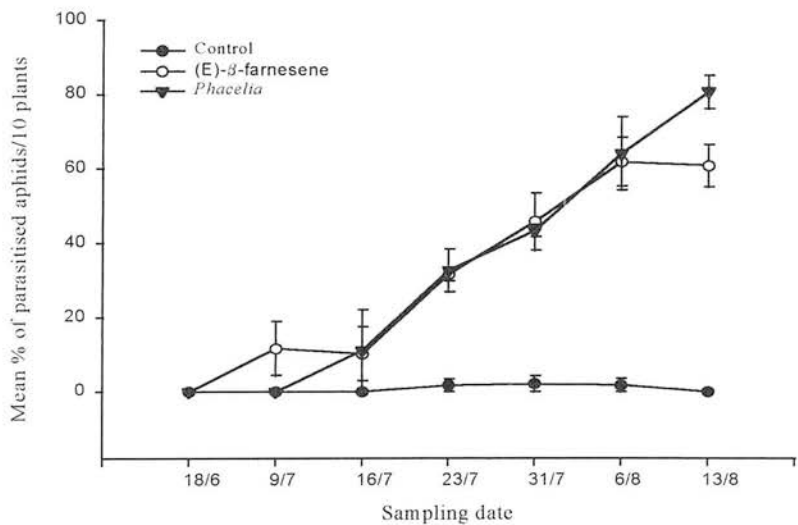


Fig. 6. Mean % of parasitised aphids (\pm SE) in areas of a winter wheat crop with control dispensers (—●—, $n = 2$), (E)-β-farnesene dispensers (—○—, $n = 4$), *Phacelia* dispensers (—▼—, $n = 3$) in 1997.

DISCUSSION

Synthesised aphid alarm pheromone (EBF) and *Phacelia* flower extract when released from dispensers in a winter wheat crop significantly alter the activity of an assemblage of carabid beetles, and increases the level of aphid parasitism by hymenopteran parasitoids. There is also a corresponding reduction in the level of aphid infestation.

Semiochemical-mediated habitat location has been reported in non-arable carabid beetle species by Evans (1988), and adults of the arable carabid beetle *Pterostichus melanarius*, which was the most common beetle found in this study, orient to olfactory cues arising directly from prey species such as crickets and blowfly larvae (Wheater, 1989) and from analogues of the pheromones of prey species such as (E)- β -farnesene (Kielty *et al.*, 1995; Kirkland & Evans unpubl.).

Polyphagous carabids have an advantage in integrated pest management programs, in that they can survive in an area when there are no pest species present by feeding on alternative prey such as Collembola (Wheater, 1989). Therefore inducing carabid beetle movement into cereal fields using semiochemicals early in the season before pest populations become established, will not lead to starvation or death of the beetles. Consequently, beetles will be present within the crop when pests such as aphids arrive in the spring/early summer, enabling the beetles to reduce the pest population when numbers are low preventing a build up to damaging levels later in the season.

There was a significant level of parasitism of aphids on plants in the vicinity of the EBF and *Phacelia* dispensers. Over 70% parasitism was recorded in the form of aphid 'mummies' being present. Parasitism in areas around the methanol control dispensers did not exceed 2.5%. By releasing EBF and *Phacelia* extract via field dispensers, movement of the parasitoids into the crop is enhanced, leading to greater levels of parasitism. Plants 70m from the crop edge did not show any differences in their level of aphid infestation or parasitism from plants closer to the edge of the field.

By using semiochemicals such as EBF and *Phacelia* flower extract to enhance the suite of predatory and parasitic insects within a crop, pests may be prevented from reaching levels economically damaging to the crop without recourse to the use of pesticides.

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